Rosa Isabel Marques Mendes Guilherme

Mestre

Influência do modo de produção, biológico e convencional, e do estado de maturação do fruto na composição e qualidade do pimento, *Capsicum annuum* L.

Dissertação para obtenção do Grau de Doutor em
Tecnologias Agroindustriais

Orientador: José Alberto Cardoso Pereira, Prof. Coordenador Principal, ESA – IPB/CIMO

Co-orientador: Fernando Henrique Reboredo, Prof. Associado com Agregação, UNL – FCT

Júri:

Presidente: Fernando José Cebola Lidon

Arguentes: Maria Dulce Carlos Antunes
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Vogais: José Alberto Cardoso Pereira
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Maria Elvira Semedo Pimentel Saraiva Ferreira
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Março, 2021

FACULDADE DE CIÊNCIAS E TECNOLOGIA
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Ao meu marido.

Recomeça...
Se puderes
Sem angústia
E sem pressa.
E os passos que deres,
Nesse caminho duro
Do futuro
Dá-os em liberdade.
Enquanto não alcances
Não descanses.
De nenhum fruto queiras só metade.

(Miguel Torga)

Agradecimentos

“…o sonho comanda a vida
E sempre que um homem sonha
O mundo pula e avança…”

Àqueles que me ajudaram a concretizar mais um sonho, ficarei para sempre grata.

O meu muito obrigada…

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À minha família. À minha irmã pela pedra basilar que é na minha vida e pelo grande exemplo que representa para mim.

Resumo

O pimento, *Capsicum annuum* L., é um fruto muito consumido e apreciado. A sua composição pode ser afetada pelo modo de produção e pelo estado de maturação dos frutos. O presente trabalho teve como objetivo obter informação acerca da produção em modo de produção biológico de pimento (variedade Entinas) na região de Coimbra, e estudar o efeito do modo de produção, produção biológica e integrada/convencional, na composição em minerais, compostos fenólicos, compostos voláteis, atividade antioxidante e caraterísticas de qualidade do pimento. Os resultados indicam que o cultivo do pimento em modo de produção biológico, em cultura de primavera/verão ao ar livre, apresenta bom desenvolvimento cultural, produtividades na ordem das 28 $t.ha^{-1}$, e que os frutos são de qualidade. A comparação dos pimentos produzidos em modo de produção biológico e convencional, em diferentes estados de maturação (verde e vermelho), permitiu constatar que os primeiros, independentemente do estado de maturação, apresentaram maiores concentrações de potássio, fósforo, cálcio e cobre, cloro, ferro e enxofre, e os segundos apresentaram concentrações de manganês e zinco superiores. Foram identificados nove compostos fenólicos: ácido cafeico, ácido clorogénico, ácido m-cumárico, ácido o-cumárico, luteolina-7-O-glucósido, miricetina, resveratrol, rutina e quercetina-3-O-rhamnosideo, e 48 compostos voláteis pertencentes a classes distintas. Os pimentos verdes produzidos de forma convencional foram os mais ricos em compostos fenólicos e com maior atividade antioxidante, bem como os que apresentaram maiores teores de compostos voláteis. Por outro lado, os pimentos produzidos no modo de produção biológico apresentaram melhor qualidade visual e tátil e menor qualidade química quando avaliados por um painel sensorial. Utilizou-se uma língua eletrónica potenciométrica que permitiu discriminar pimentos de acordo com o modo de produção e estado de maturação dos frutos. No presente trabalho os pimentos provenientes do modo de produção convencional apresentaram, no geral, melhor qualidade e melhores caraterísticas químicas.

Palavras-chave – *Capsicum annuum* L.; composição química; maturação do fruto; modo de produção biológico/convencional; pimento; qualidade.

Abstract

Sweet pepper, *Capsicum annuum* L., is a widely consumed and appreciated fruit. Its composition can be affected by the production system and ripeness stage of fruits. This work aims to obtain information about pepper (variety Entinas) in organic production in the region of Coimbra, and to study the effect of production system, organic and integrated / conventional production, in its mineral, phenolic and volatile composition, antioxidant activity and quality characteristics. The results indicated that the production of sweet pepper in organic production, in spring / summer open-air conditions, presents good cultural development, productivity around 28 $t.ha^{-1}$, and that the fruits are of good quality. The comparison of peppers produced in organic and conventional production systems, in different ripening stages (green and red), showed that the first ones, regardless of their ripeness, had higher concentrations of potassium, phosphorus, calcium and copper, chlorine, iron and sulphur, and the seconds showed higher concentrations of manganese and zinc. Nine phenolic compounds were identified: caffeic acid, chlorogenic acid, m-cumaric acid, o-cumaric acid, luteolin-7-O-glucoside, myricetin, resveratrol, rutin and quercetin-3-O-rhamnoside, and 48 volatile compounds belonging to distinct classes. Green peppers produced in the conventional system were the richest in phenolic compounds and had the highest antioxidant activity, as well as those with the highest levels of volatile compounds. On the other hand, peppers produced in organic system showed better visual and tactile quality and lower chemical quality when evaluated by a trained sensory panel. An electronic potentiometric tongue was used, which allowed discriminate peppers according to the system of production and fruits ripeness. In this study, peppers from the conventional production system showed, in general, better quality and better chemical characteristics.

Keywords: *Capsicum annuum* L.; chemical composition; fruit ripening; organic/conventional production; quality; sweet pepper.

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Introdução, enquadramento e objetivos

Capítulo 1

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1.1. Introdução

Nas últimas décadas tem-se notado uma preocupação crescente por parte dos consumidores, pela procura de alimentos menos calóricos, mais ricos em determinados nutrientes e compostos bioativos, produzidos de forma mais sustentável e sem recurso a produtos químicos de síntese. Nesta ótica, as hortaliças, frutos e vegetais têm vindo a ganhar uma particular atenção, bem como os produtos oriundos do modo de produção biológico, produzidos sem recurso a fertilizantes e pesticidas de origem química, que têm ganho popularidade e representam um segmento importante do mercado com tendência para grande crescimento.

Tem havido também uma atenção crescente no estilo de vida dos povos dos países Euro-Mediterrânicos e na sua dieta, a dieta mediterrânica. De facto, diferentes estudos epidemiológicos têm demonstrado que este tipo de dieta é mais saudável que as restantes e apresenta um impacto muito benéfico na saúde humana (Cena & Calder, 2020). Para além do consumo de cereais, este tipo de dieta é muito rico em frutos e vegetais (Cena & Calder, 2020; Leri et al., 2020), consumidos em diferentes preparações culinárias desde crus, cozidos e assados, e em diferentes formas, frescos, secos e secados o que afeta a sua composição (Rashmi & Negi, 2020).

Os vegetais e frutos são fontes naturais de compostos com elevada atividade biológica, destacando-se a antioxidante. Entre os compostos de maior interesse podem referir-se, entre outros, os minerais, os compostos fenólicos, as vitaminas, e os compostos aromáticos que têm sido positivamente relacionados com os efeitos saudáveis de uma alimentação rica nestes alimentos. Os compostos fenólicos, têm merecido particular interesse na comunidade científica, uma vez que podem contribuir para a redução do stress oxidativo prevenindo a formação de radicais livres como espécies reativas de oxigénio ou azoto geradas pelo metabolismo celular e que provocam alterações ao nível celular, atuando os compostos fenólicos como protetores e que variados estudos demonstraram o seu efeito benéfico na saúde (Leri et al., 2020).

Também no que respeita ao modo de produção biológico, tem sido entendido por parte dos consumidores como um modo de produção mais amigo do ambiente, em que não é permitida a utilização de produtos químicos de síntese, fazendo com que estes produtos sejam considerados mais seguros e com melhor qualidade por parte de quem os adquire. As áreas destinadas a este modo de produção têm vindo a aumentar consistentemente de forma a dar resposta a uma procura crescente por parte dos mercados.

De seguida faz-se um breve enquadramento acerca do pimento, fruto objeto deste trabalho, à sua classificação, origem e composição, bem como aos modos de produção hortícola e a importância que tem no mundo e em Portugal.

1.2. Enquadramento

1.2.1. O pimento

O pimenteiro, com o nome científico de *Capsicum annuum* L., é uma espécie domesticada em que os seus frutos, os pimentos, são muito consumidos e apreciados. O género *Capsicum* faz parte da grande família das Solanaceae. Esta família integra mais de 90 géneros e 2 500 espécies de plantas, e inclui vegetais comercialmente importantes, como a batata, a beringela, o pimento e o tomate (Tripodi & Kumar, 2019). O género *Capsicum* abarca mais de 30 espécies, das quais cinco domesticadas, nomeadamente *C. annuum*, *C. frutescens*, *C. chinense*, *C. baccatum* e *C. pubescens*. *C. annuum* é a que apresenta maior variação de tamanho, forma e cor dos frutos (Guilar et al., 2009).

Considera-se que o centro de origem do género *Capsicum* (*Capsicum* spp) é o continente Americano, sobretudo a área que compreende o México, a América Central e o norte da América do Sul (Zhigila et al, 2014). Este género engloba uma elevada diversidade de características entre si, como sejam a arquitetura da planta, a morfologia da flor, a tipologia do fruto, as cores do fruto, a pungência e as características qualitativas (Guilar et al., 2009).

O pimento é conhecido por diversos nomes em diferentes partes do mundo atribuindo-se a Cristóvão Colombo (1493) a sua disseminação na Europa sendo, hoje, cultivado em todo o mundo pelos seus frutos. O género *Capsicum*, conhecido como "pimentão vermelho", "pimento", "pimenta vermelha quente", "tabasco", "paprica", "pimenta de Caiena" é extremamente versátil sendo os frutos consumidos frescos ou cozinhados e em diferentes estados de maturação (verdes, vermelhos) e usados não apenas como vegetais mas, também, como aroma em produtos alimentícios, produtos farmacêuticos e cosméticos (Bosland & Votava, 2012).

Em 2018, a nível mundial, a área destinada à produção de pimento rondava os dois milhões de hectares (Tabela 1), sendo a China o maior produtor mundial com uma produção de 18 184 711 de toneladas numa área de 769 078 hectares (FAOSTAT 2020).

Tabela 1. Dados relativos, área produção e produtividade, da cultura do pimento no Mundo em 2018 (FAOSTAT 2020)

Região	Área (x 1000 ha)	Produção (x 1000 t)	Produtividade ($t.ha^{-1}$)
Europa	106,9	3 219,4	30,1
União Europeia	62,8	2 588,4	41,2
Africa	311,8	3 478,1	11,2
América	243,0	5 029,4	20,7
Oceânia	2,2	53,2	24,1
Asia	1 326,5	24 991,3	18,8
Mundo	1 990,4	36 771,5	18,5

Por sua vez, na União Europeia, o maior produtor de pimento foi a Espanha, com uma produção de 1 275 457 t e uma área de 20 580 hectares (FAOSTAT 2020).

Em Portugal, os últimos dados estatísticos que se referem ao ano de 2018, apontam para uma área destinada à cultura do pimento de 926 ha, com uma produção de 38 137 t, e uma produtividade de 41,2 $t.ha^{-1}$ (INE, 2020). A produção comercial de pimento ocorre sobretudo nas regiões do Ribatejo e Oeste, Entre-Douro e Minho, Beira Litoral e Algarve, sendo maioritariamente produzido em estufa (GPP, 2020).

Em climas temperados o pimenteiro é uma planta anual, apesar de na sua região de origem ser bienal. De porte ereto, tem crescimento indeterminado e pode atingir 1,5 m de altura. Apresenta ramificação lateral nos primeiros 8 a 10 nós, tornando-se esta dicótoma a partir do aparecimento da primeira flor produzindo, a partir daí, uma ou mais flores em cada nó. As folhas são inteiras, peninérveas, ovadas ou lanceoladas, glabras e com inserção alterna. Apresenta flores normalmente solitárias, completas, hermafroditas, de corola simpétala rodada, 5-7 pétalas e, geralmente, brancas. A polinização é maioritariamente autogâmica. O fruto é uma baga de forma e tamanho variáveis e com coloração variável de acordo com a variedade e estado de maturação (Almeida, 2006).

A temperatura ótima de desenvolvimento do pimenteiro situa-se entre os 18 a 25ºC, com humidade relativa de 50 a 70%. Esta espécie é muito sensível às geadas, em especial às primaveris, o que condiciona as plantações precoces ao ar livre. Altas temperaturas e baixa percentagem de humidade relativa, provocam a queda dos botões florais e a formação de frutos de pequeno tamanho. Com baixa luminosidade os entrenós dos caules alongam-se e a planta torna-se débil e floresce menos. Em termos de solos esta planta prefere solos com texturas arenosas ou francas, profundos e bem drenados. Os solos argilosos são de evitar pois tendem a aquecer lentamente. Sensibilidade moderada à salinidade: intervalo ótimo de pH 6,0 – 7,0 e apresenta moderada tolerância à acidez (Almeida, 2006).

Em Portugal, o pimenteiro apresenta um ciclo cultural caraterístico de Primavera/Verão. A reprodução ocorre por semente, sendo as plântulas transplantadas 6 a 7 semanas após sementeira. Passadas 7 a 9 semanas após a instalação tem início o ciclo produtivo. De acordo com Lopes & Simões (2006), os principais estados fenológicos do pimenteiro são: 1) Plantas com 12-15 cm e 5 a 10 folhas; 2) Pleno desenvolvimento, a planta está instalada e apresenta completo desenvolvimento de folhas; 3) Início da floração, aquando do aparecimento dos primórdios florais; 4) Plena floração, flores visíveis; 5) Vingamento e crescimento dos frutos e 6) Colheita, que pode ser feita em verde ou na fase final de maturação (verde, vermelho, amarelo, laranja) de acordo com as caraterísticas da variedade cultivada. Os estados fenológicos constituem indicadores ideais do impacto de mudanças locais e globais no clima e na biosfera da Terra (Meier et al., 2009). O pimento apresenta uma produção escalonada que se pode prolongar no tempo durante 4-6 semanas e que estão dependentes da variedade, do fim para que se destina a produção e das condições climáticas.

Existe um grande número de cultivares de pimento, *C. annuum* que podem ser agrupadas em dois grupos hortícolas (Almeida, 2006), nomeadamente Grupo *Grossum* que inclui os tipos designados por pimento, pimentão e paprika; e Grupo *Longum* – inclui as malaguetas (sin. Jindungo ou pimenta de Caiena). E os seus frutos podem ser agrupados em diferentes tipos de acordo com as suas caraterísticas morfológicas. A maioria das cultivares economicamente relevantes são do tipo Bell.

Os frutos adultos podem apresentar diferentes colorações de acordo com o estado de maturação do fruto. Os carotenoides são os pigmentos responsáveis pela cor vermelha, sendo a capsatina, a capsorubina e a criptoxantina os pigmentos mais abundantes nos pimentos. O fruto é oco encontrando-se no seu interior as sementes de forma achatada, ovoides e com 3 a 5 mm de comprimento (Almeida, 2006).

A composição média dos pimentos crus encontra-se na Tabela 2, de acordo com a tabela de composição nutricional publicada pelo Instituto Nacional de Saúde Pública Dr. Ricardo Jorge.

Tabela 2. Composição média dos frutos de pimento cru, valores expressos em unidades por 100g de parte edível (INSA, 2020).

COMPONENTES	por 100 g (parte edível)	por porção recomendada
ENERGIA		
Energia (kcal)	22	40
Energia (kJ)	92	166
MACROCONSTITUINTES		
Água (g)	92,8	167
Proteína (g)	1,6	2,9
Gordura total (g)	0,6	1,1
Total de Hidratos de Carbono disponíveis (g)	2,7	4,9
Total de Hidratos de Carbono expresso em monossacáridos (g)	2,7	4,9
Mono+dissacáridos (g)	2,5	4,5
Ácidos orgânicos (g)	0	0
Álcool (g)	0	0
Amido (g)	0,1	0,2
Oligossacáridos (g)	0,1	0,2
Fibra alimentar (g)	2,0	3,6
ÁCIDOS GORDOS		
Ácidos gordos saturados (g)	0,1	0,2
Ácidos gordos monoinsaturados (g)	0	0
Ácidos gordos polinsaturados (g)	0,3	0,5
Ácidos gordos trans (g)	0	0
Ácido linoleico (g)	0,3	0,5
COLESTEROL		
Colesterol (mg)	0	0
VITAMINAS		
Vitamina A total (equivalentes de retinol) (ug)	217	391
Caroteno (mg)	1300	2340
Vitamina D (ug)	0	0
a-tocoferol (mg)	0,80	1,44
Tiamina (mg)	0,020	0,036
Riboflavina (mg)	0,010	0,018
Equivalentes de niacina (mg)	0,80	1,44
Niacina (mg)	0,60	1,08
Triptofano/60 (mg)	0,20	0,36
Vitamina B6 (mg)	0,31	0,56
Vitamina B12 (ug)	0	0
Vitamina C (mg)	90	162
Folatos (ug)	28	50
MINERAIS		
Cinza (g)	0,40	0,72
Sódio (Na) (mg)	4,0	7,2
Potássio (K) (mg)	120	216
Cálcio (Ca) (mg)	9,0	16,2
Fósforo (P) (mg)	24	43
Magnésio (Mg) (mg)	10	18
Ferro (Fe) (mg)	0,6	1,1
Zinco (Zn) (mg)	0,2	0,4

1.2.2. Modos de produção hortícola

Na atualidade, em produção agrícola são reconhecidos dois modos de produção, o modo de produção biológico, vulgarmente designado apenas de agricultura biológica, e o modo de produção integrado que, muitas vezes, também se apelida de convencional.

O modo de produção biológico, é um sistema global de gestão das explorações agrícolas e de produção de géneros alimentícios, que combina as melhores práticas ambientais, um elevado nível de biodiversidade, a preservação dos recursos naturais, a aplicação de normas exigentes em matéria de bem estar dos animais e método de produção em sintonia com a preferência de certos consumidores de produtos obtidos utilizando substâncias e processos naturais (Reg. (CE) N.o 834/2007 do Conselho de 28 de junho de 2007). Entre variados aspetos considerados no modo de produção biológico, salientam-se a proibição de utilização de fertilizantes minerais azotados e da utilização de pesticidas de síntese (consideradas apenas algumas exceções).

A Federação Internacional dos Movimentos da Agricultura Orgânica (IFOAM,2020) realça que o modo de produção biológico, é um sistema de produção que promove a saúde dos solos, ecossistemas e pessoas. Baseia-se em processos ecológicos, biodiversidade e ciclos adaptados às condições locais, em vez do uso de fatores de produção com efeitos adversos. Combina tradição, inovação e ciência de modo a ser benéfica para o espaço partilhado, promove relacionamentos justos e uma boa qualidade de vida para todos os envolvidos.

De acordo com o Instituto de Pesquisa em Agricultura Orgânica - FiBL (Willer et al., 2020) a área mundial em agricultura biológica situou-se, em 2018, nos 71,5 milhões de hectares representando um crescimento de 2,9% ou 2 milhões de hectares em relação a 2017 correspondendo o número de produtores biológicos a 2,8 milhões, em 2018, num total de 186 países. A mesma fonte refere que o mercado global de alimentos biológicos ultrapassou os 100 bilhões de dólares pela primeira vez em 2018 (quase 97 bilhões de euros). Os Estados Unidos foram o mercado líder, com 40,6 bilhões de euros, seguidos pela Alemanha (10,9 bilhões de euros) e França (9,1 bilhões de euros). Em 2018, muitos dos principais mercados continuaram a apresentar taxas de crescimento de dois dígitos, e o mercado biológico francês cresceu mais de 15%. Os consumidores dinamarqueses e suíços foram os que gastaram mais em alimentos biológicos (312 euros per capita).

Em Portugal, de acordo com a Direção Geral de Agricultura e Desenvolvimento Rural (DGADR) a área de superfície agrícola útil (SAU) dedicada à agricultura biológica, em 2019, situou-se acima dos 7% correspondendo a 273 158 hectares, com 5 637 produtores. Registou-se, ainda, uma área de 20 055 hectares em conversão.

A produção integrada é um sistema agrícola de produção de alimentos e de outros produtos alimentares de alta qualidade, com gestão racional dos recursos naturais e privilegiando a utilização dos mecanismos de regulação natural em substituição de fatores de produção, contribuindo, deste modo, para uma agricultura sustentável (Decreto-Lei n.º 256/2009 de 24 de setembro; Art.º 7º). Este sistema de produção baseia-se num conjunto de boas práticas que obedecem aos seguintes princípios: 1) regulação do ecossistema, importância do bem-estar dos animais e preservação dos recursos naturais; 2) exploração agrícola no seu conjunto, como a unidade de implementação da produção integrada; 3) atualização regular dos conhecimentos dos agricultores sobre produção integrada; 4) manutenção da estabilidade dos ecossistemas agrários; 5) equilíbrio do ciclo dos nutrientes, reduzindo as perdas ao mínimo; 6) preservação e melhoria da fertilidade intrínseca do solo; 7) fomento da biodiversidade; 8) entendimento da qualidade dos produtos agrícolas como tendo por base parâmetros ecológicos, assim como critérios usuais de qualidade, externos e internos; 9) proteção das plantas tendo obrigatoriamente por base os objetivos e as orientações da proteção integrada e 10) minimização de alguns dos efeitos secundários decorrentes das atividades agrícolas.

Na produção integrada pode recorrer-se à utilização de pesticidas de síntese de acordo com os princípios da proteção integrada, assim como à utilização de fertilizantes sintéticos.

Os modos de produção descritos enquadram-se nos modos de produção sustentáveis uma vez que ambos apresentam preocupações de foro ambiental nomeadamente com a melhoria da fertilidade do solo e o incremento da biodiversidade. Estes aspetos vão ao encontro das principais preocupações nacionais e internacionais refletidas no plano denominando por "Transformando nosso mundo: a AGENDA 2030 para o desenvolvimento sustentável" aprovado na cimeira da Organização das Nações Unidas (ONU) em 2015, que apresenta os 17 Objetivos de Desenvolvimento Sustentável (ODS), sendo o 12º sobre produção e consumo sustentáveis.

De acordo com a FAO (Organização das Nações Unidas para Agricultura e Alimentação) a população mundial deverá aumentar para 9 mil milhões de pessoas em 2050. Espera-se que algumas das mais altas taxas de crescimento populacional ocorram em áreas que dependem pesadamente no setor agrícola (agricultura, pecuária, silvicultura e pesca) e que têm altos níveis de insegurança alimentar. O crescimento no setor agrícola é uma das formas mais eficazes de reduzir a pobreza e alcançar a segurança alimentar.

No início do Século XXI a FAO registava 800 milhões de pessoas a viver em situação de insegurança alimentar. A previsão de escassez de alimentos para as próximas décadas, tem vindo a provocar um forte impacto ambiental na produção de alimentos, com consumos exagerados de recursos naturais e perda da biodiversidade, o que deverá ser evitado. Esta situação está a acontecer em todo o mundo, com mais peso nas regiões de maior densidade populacional. Assim, têm surgido políticas internacionais e nacionais de âmbito agrícola e alimentar, que indicam caminhos a seguir e alertam e consciencializam os cidadãos para estes novos desafios.

Ainda em 2015, foi publicado pela União Europeia o Plano de Ação para a Economia Circular, transposto para a legislação portuguesa em 2017. A Agência Europeia do Ambiente publicou o relatório 4/2019, sobre a Adaptação do Sector Agrícola na Europa às Alterações Climáticas, com os seguintes destaques para as culturas hortícolas: aplicação de rotações culturais; aumento da eficiência da rega; produção sustentável em culturas protegidas e alteração dos calendários culturais. Para executar a Agenda 2030 e concretizar os ODS, foi também publicado pela União Europeia em 2019, o Pacto Ecológico Europeu que adotou a Estratégia do Prado ao Prato, em defesa de um sistema alimentar mais sustentável e onde a redução do uso de pesticidas na agricultura, a limitação de excesso de nutrientes nas culturas e o aumento de cerca de 25% de produção biológica, são algumas das soluções apontadas.

Especificamente sobre agricultura biológica, referencia-se a Estratégia Nacional para a Agricultura Biológica (Resolução do Conselho de Ministros n.º 110/2017) e em 2018, o Regulamento (EU) 848 do Parlamento Europeu e do Conselho, relativo à Produção Biológica e à rotulagem dos produtos biológicos, que apontam metas ambiciosas para o setor das quais se destaca a duplicação de área neste modo de agricultura.

Em 2019, a Resolução do Conselho de Ministros n.º 107/2019, aprovou o Roteiro para a Neutralidade Carbónica 2050 (RNC 2050), adotando o compromisso de alcançar a neutralidade carbónica em Portugal até 2050, que se traduz num balanço neutro entre emissões de gases com efeito de estufa (GEE) e o sequestro de carbono pelo uso do solo e florestas. O contributo da agricultura biológica para a diminuição da emissão de CO_2 será de 48% a 60% devido à não utilização de fertilizantes de síntese química.

A procura por alimentos biológicos tem aumentado nas últimas décadas (Willer et al., 2020) apresentando-se como principais motivações de compra aspetos relacionados com benefícios que estes produtos trazem à saúde e ao meio ambiente. Os consumidores consideram, ainda, que estes alimentos são mais nutritivos e têm melhor sabor.

1.3. Objetivos

Considerando que o pimento é um dos vegetais muito consumido quer em Portugal quer no mundo, e que é um dos componentes da dieta Mediterrânica, e também porque existe uma procura crescente por produtos oriundos de modo de produção biológico, a presente tese de doutoramento teve como objetivo geral obter informação acerca da produção de pimento (*C. annum* variedade Entinas) na região de Coimbra, nomeadamente quando produzido em modo de produção biológico, e comparar os frutos produzidos, em diferentes estados de maturação, com frutos produzidos em produção integrada (convencional). Nesse sentido a tese está organizada em sete capítulos.

No primeiro capítulo faz-se uma introdução geral e enquadramento sobre a temática em estudo, abordando aspetos acerca das caraterísticas da planta e dos modos de produção em que é produzida de forma comercial.

No segundo capítulo apresentam-se os principais resultados obtidos do acompanhamento, ao longo de duas campanhas de produção em dois anos consecutivos, de uma cultura de pimento variedade Entinas quando produzido em modo de produção biológico.

No terceiro capítulo compara-se a composição em macronutrientes e micronutrientes de pimentos em dois estados de maturação, verde e vermelho, quando produzidos em modo de produção biológico e em agricultura convencional.

No quarto capítulo, procedeu-se ao estudo da fração fenólica e da atividade antioxidante de extratos obtidos de frutos de pimento em dois estados de maturação (verde e vermelho) e oriundos de dois sistemas de produção, modo de produção biológico e convencional.

No quinto capítulo uma vez que a fração volátil é um dos aspetos que muito influi na qualidade de vegetais, estuda-se essa fração em pimentos frescos em dois estados de maturação (verdes e vermelhos) e dois sistemas de produção (modo de produção biológico e convencional).

No capítulo 6, procede-se à comparação de caraterísticas qualitativas do ponto de vista físico-químico e sensorial de pimentos em três estados de maturação, verde, mudança de cor e vermelho, quando produzidos em ambos os modos de produção (modo de produção biológico e convencional). Neste capítulo procede-se ainda à utilização de uma língua eletrónica como ferramenta complementar com potencial na caraterização de propriedades de pimentos.

Por fim, no capítulo 7, faz-se uma súmula das principais conclusões do presente trabalho.

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Capítulo 1

Capítulo 2

Crescimento e produção do pimenteiro, *Capsicum annuum* L., variedade Entinas, na região de Coimbra

Capítulo 2

Resumo

O pimenteiro, *Capsicum annuum* L., é uma planta com crescimento indeterminado, de caule ereto, e que vegeta na primavera-verão. Os seus frutos, os pimentos, são dos vegetais mais consumidos e apreciados no mundo. O desenvolvimento, crescimento e produtividade do pimenteiro são influenciados por diversos fatores, entre os quais destacando-se as condições agroecológicas da região, a variedade e o sistema de produção. Neste sentido, este trabalho teve por objetivo obter dados acerca do ciclo de desenvolvimento e produtividade da cultura na região de Coimbra quando produzida em modo de produção biológico. Para tal, durante dois anos consecutivos (2018 e 2019), foi instalado um ensaio, ao ar livre, em Bencanta, Coimbra, numa parcela pertencente à Escola Superior Agrária certificada para o modo de produção biológico. As plantas, da variedade Entinas, foram instaladas no campo em meados de maio, a 21 em 2018 e a 15 em 2019, com uma densidade de plantação de 22 222 plantas/ha e 24 242 plantas/ha, respetivamente em 2018 e 2019. Durante o ciclo cultural, em 30 plantas selecionadas aleatoriamente na parcela, foram anotados os estados fenológicos (transplantação, pleno desenvolvimento, floração, vingamento e maturação/colheita) e avaliada a produtividade da cultura (número de frutos, peso do fruto e produção por hectare). As plantas apresentaram um desenvolvimento normal, tendo as primeiras flores surgido no início de julho, e a meados do mesmo mês foram observados os primeiros frutos vingados. O máximo de floração registou-se no final de julho e os primeiros frutos foram considerados maduros no início de agosto. Nas condições agroecológicas em que decorreu o ensaio, desde a instalação das plântulas no campo até à última colheita decorreram cerca de 150 dias. A produção acumulada foi de 28 toneladas por hectare o que é considerado aceitável para o modo de produção biológico.

Capítulo 2

2.1. Introdução

O género *Capsicum*, pertencente à família das solanáceas, inclui diferentes espécies com grande importância económica. As espécies deste género, que inclui os pimentos e as malaguetas, são oriundas das regiões tropicais e temperadas da América, tendo sido introduzidas na Península Ibérica, em finais do século XV, de onde se disseminaram para o resto da Europa, Africa, Índia e China (Ferrão, 1992; Delgado & Diogo, 2015). A espécie *Capsicum annuum* L., onde se incluem os pimentos doces, é a principal espécie cultivada deste género e um dos vegetais mais consumidos (Pikergill, 1997).

Em Portugal, a cultura do pimenteiro encontra-se disseminada por todo o País. Ainda que em muitas regiões a sua produção ocorra maioritariamente em pequenas hortas e quintais, sobretudo para consumo familiar e sem expressão em termos de comercialização, é nas regiões do Ribatejo e Oeste, Entre-Douro e Minho, Beira Litoral e Algarve que se concentra a produção comercial, que ocorre maioritariamente em sistemas protegidos (estufa), apesar de em algumas regiões como a Beira Litoral a produção ao ar livre ter uma expressão importante (GPP, 2020).

O pimenteiro, é uma planta bienal, que em climas temperados é cultivada como anual. Esta planta inicialmente assume uma postura herbácea, contudo, com a idade, o seu caule torna-se lenhoso. Tem porte ereto e apresenta crescimento indeterminado podendo atingir 1,5 m de altura. O sistema radicular é aprumado. Apresenta ramificação lateral nos primeiros 8 a 10 nós, tornando-se esta dicótoma a partir do aparecimento da primeira flor produzindo, a partir daí, uma ou mais flores em cada nó. As folhas são inteiras, peninérveas, ovadas ou lanceoladas, glabras e com inserção alterna. Normalmente solitárias, as flores são completas, hermafroditas, de corola simpétala rodada, 5-7 pétalas e, geralmente, brancas. Polinização maioritariamente autogâmica. O fruto, de cor que varia à maturação de acordo com as variedades de verde, amarelo, púrpura, evoluindo até amarelo, laranja, vermelho ou mistura destas quando maduro, é uma baga com forma e tamanho muito variáveis. O fruto é oco encontrando-se no seu interior as sementes de forma achatada, ovoides e com 3 a 5 mm de comprimento (Almeida, 2006).

A multiplicação do pimenteiro ocorre apenas através de semente (Pádua et al.., 1984), e o seu desenvolvimento e fenologia podem ser influenciados por diferentes fatores (Paulus et al.., 2015) como a variedade usada, as caraterísticas agroclimáticas da região onde é cultivado, bem como aspetos ligados ao modo de produção, como sejam a densidade de plantas, a disponibilidade de água e fertilizantes, o ataque de pragas e doenças, entre outros. Neste sentido, neste capítulo pretendeu obter-se dados da fenologia e produção de pimenteiro, da variedade Entinas, produzido em modo de produção biológico, na região agroclimática de Coimbra. As observações incidiram num campo ao ar livre por ser um dos sistemas mais utilizados em modo de produção biológico e na região.

2.2. Material e métodos

2.2.1. Caraterização do local

Os trabalhos de campo decorreram em dois anos consecutivos, 2018 e 2019, na Região do Baixo Mondego, numa parcela da Escola Superior Agrária de Coimbra (ESAC), designada por Caldeirão. A parcela escolhida encontra-se inserida na área certificada em Modo de Produção Biológico, desde 2009, onde são obrigatoriamente cumpridos os requisitos estabelecidos no Regulamento (CE) N.o 834/2007 do Conselho de 28 de Junho de 2007 (Comissão Europeia, 2007), relativo à produção biológica e à rotulagem dos produtos biológicos.

No início do ensaio, em maio de 2018, e no início do ano seguinte, em fevereiro de 2019, procedeu-se à análise de solos da parcela destinada aos ensaios. A análise foi feita no Laboratório de solos da Escola Superior Agrária de Coimbra e seguiu os procedimentos analíticos de rotina em uso no laboratório. Observou-se que os valores são muito semelhantes em ambos os períodos de análise. O solo apresenta uma textura média, com um teor em matéria fina de cerca de 78% (78,04-78,23%), com baixo teor de matéria orgânica (1,3%) e pH (H_2O) pouco ácido com valor de 6,4 (6,3-6,5). A parcela apresentava altos níveis de potássio (K_2O) extraível de (122-150 mg/kg^{-1}), teores médios de fósforo (P_2O_5) extraível (77-84 mg/kg^{-1}), baixos teores de Ca2+ (4,0 meCa^{2+}/100g) e Mg^{2+} (0,80 meMg^{2+}/100g) e uma relação de Ca/Mg (5) alta. Os níveis de Cu (3,8 mg Cu/Kg), Zn (2,3 mg Zn/Kg) e Mn (17 mg Mn/Kg) foram considerados médios e o de Fe (109 mg Fe/Kg) muito alto.

O pimento prefere solos de textura arenosa ou franco-arenosa, ricos em matéria orgânica (entre 2 a 4%), pH entre 6,0 e 7,0 (sendo muito sensível à acidez do solo). Apresenta elevada sensibilidade às situações de carência em nutrientes secundários e micronutrientes, particularmente Ca e Mg (LQARS, 2006). Produz bem em solos com uma condutividade elétrica <0,4 dS/m, desenvolvendo-se melhor em solos bem drenados com boas características de retenção de água.

2.2.2. Condições ambientais

Os dados climáticos foram registados na estação agrometeorológica da ESAC (Lat.: 40º 12’ 59’’N; Long.: 8º 26’ 57’’ W).

Segundo o método de Thornthwaite o clima de Coimbra/Bencanta é do tipo B2 B'2 s a', clima pouco húmido, mesotérmico, com moderada deficiência de água no Verão e com nula ou pequena concentração da eficiência térmica na estação quente.

Os dados climáticos respeitantes aos períodos em que decorreram os ensaios, nomeadamente temperatura média, humidade relativa média e precipitação encontram-se sistematizados na Figura 2.1.

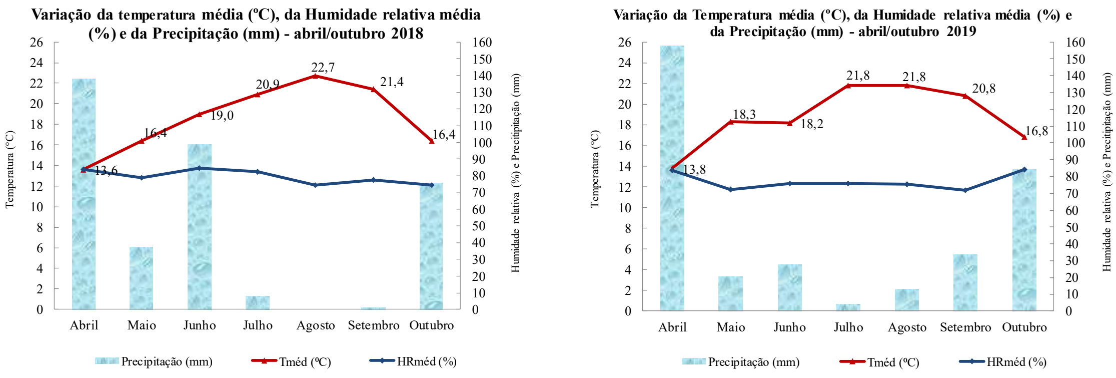

Figura 2.1. Variação da temperatura média (ºC), da humidade relativa média (%) e da precipitação (mm) no decorrer do ensaio de abril/outubro 2018 (esquerda) e de 2019 (direita) em Bencanta, Coimbra.

A temperatura ótima de desenvolvimento do pimento situa-se entre os 18 a 25ºC, com humidade relativa de 50 a 70%. É muito sensível às geadas, em especial às primaveris, o que condiciona as plantações precoces ao ar livre. Altas temperaturas e baixa percentagem de humidade relativa, provocam a queda dos botões florais e a formação de frutos de pequeno tamanho. Com baixa luminosidade os entrenós dos caules alongam-se e a planta torna-se débil e floresce menos.

2.2.3 Instalação da cultura

Em ambos os anos, foram adquiridas plântulas de pimento, *Capsicum annuum* L., variedade. Entinas, num viveiro comercial BIOBROTAR – Viveiros de plantas Biológicas e Serviços, Lda. Em 2018, as plantas foram instaladas no solo a 21 de maio com um compasso de 0,75 m x 0,60 m, numa área total de 270 m^2, ao que corresponde uma densidade de 22 222plantas/hectare. Em 2019, a plantação ocorreu a 15 de maio, e o compasso utilizado foi de 0,75 m x 0,55 m, numa área total de 270 m^2 e com uma densidade de 24 242plantas/hectare. O sistema de rega instalado foi de gota a gota.

A preparação do solo para a instalação da cultura teve início com o destroçamento da camada vegetal superficial e com a distribuição de 800 kg de estrume de equino (29,6 t/ha), seguida de incorporação. O estrume de equinos e de acordo com Santos (2015), apresenta um teor de macronutrientes principais (g kg^{-1} da matéria original) de 5,8 de N, 2,8 de P_2O_5 e de 5,3 K_2O.

O sistema de rega instalado foi de gota a gota com um caudal de 2,66l/s e uma dotação de 35,47mm/h, pelo que se obedeceu a um intervalo entre regas de 2 e 3 dias, concretamente à 2ª, 4ª e 6ª, durante 20 min, sempre que não ocorreu precipitação. A cultura esteve no campo desde a plantação (meados de maio) a finais de outubro. Durante este período efetuaram-se as regas descritas e manteve-se a superfície livre de infestantes com recurso à monda manual (utilização de sacho) na linha e à monda mecânica na entrelinha através da utilização de uma moto-enxada, sempre que necessário. Não foram efetuadas fertilizações e não houve aplicação de qualquer tratamento fitossanitário uma vez que não se manifestaram problemas sanitários no decorrer do ciclo.

2.2.4. Acompanhamento do estado fenológico da cultura

O pimento tem um ciclo cultural característico de Primavera/Verão. O seu ciclo produtivo tem início cerca de 7 a 9 semanas após a instalação. Para o registo dos estados fenológicos da cultura adotou-se a escala referida por Lopes & Simões (2006), avaliando (Figura 2.2.):

1) *Planta com 12-15 cm de altura*, corresponde ao período em que as plantas são transplantadas para local definitivo e acontece quando as plantas apresentem 12-15 cm de altura e 5 a 10 folhas;
2) *Pleno desenvolvimento*, corresponde ao completo desenvolvimento de folhas;
3) *Início da floração*, aquando do aparecimento dos primórdios florais;
4) *Plena floração*, flores visíveis;
5) *Vingamento* e crescimento dos frutos e
6) *Maturação/colheita*, que pode ser feita em verde ou na fase final de maturação (verde, vermelho, amarelo, laranja) de acordo com as caraterísticas da variedade cultivada.

Por uma questão de facilidade de avaliação, e visto poder haver alguma dificuldade de avaliação, os estados 3 e 4 foram incluídos num único estado designado de floração.

A avaliação do desenvolvimento vegetativo da cultura decorreu nos dois anos em estudo, com periodicidade semanal, desde a plantação até à maturação, pela observação de 30 plantas. Para facilidade de registos optou-se pela seguinte escala: ≤10%; 10-25%; 25-50%; 50-75% e ≥75% que correspondia ao estado de desenvolvimento maioritário no período de avaliação.

Figura 2.2. Estados fenológicos do pimenteiro, *Capsicum annuum* L.. por ordem de sequência 1) Planta com 12-15 cm de altura, 2) Pleno desenvolvimento, 3) Início da floração, 4) Plena floração, 5) Vingamento e crescimento dos frutos e 6) Maturação/colheita

2.2.5. Avaliação da produção

Para efetuar a avaliação da produção, a partir do estado fenológico 25-50% de maturação, procedeu-se à seleção aleatória de 30 plantas, onde se procedeu à colheita dos frutos e se registou o número de frutos colhidos e o peso total dos frutos. As cinco colheitas realizadas ocorreram de 19 de agosto a 10 de outubro com intervalos de aproximadamente 15 dias.

2.3. Resultados e discussão

2.3.1. Ciclo vegetativo e reprodutivo

O acompanhamento dos estados fenológicos das plantas é um indicador de grande importância na avaliação da adaptação de variedades/cultivares a determinadas condições locais, e também na avaliação do impacto de mudanças locais e globais no clima e na biosfera da Terra (Meier et al., 2009). O pimento apresenta uma produção escalonada que se pode prolongar no tempo e que está dependente da variedade, do fim para que se destina a produção e das condições climáticas.

Na Figura 2.3. procede-se à representação sequencial da ocorrência dos estados fenológicos do pimenteiro, variedade Entinas, em Coimbra, nos dois anos de estudo, isto é 2018 e 2019. Como referido no material e métodos, para evitar a ocorrência de erros, e uma vez que ocorriam simultaneamente diferentes estados dentro da mesma planta, para a floração optou-se por considerar ambos os estados em apenas um.

As alterações ao nível da fenologia nos diferentes anos de observação foram mínimas, o que poderá estar relacionado por um lado, com a grande proximidade de datas de transplantação, tendo ocorrido com apenas 5 dias de diferença de 2018 para 2019 e, por outro lado, com as condições atmosféricas muito semelhantes verificadas durante o primeiro período de desenvolvimento da cultura em que se registaram valores de temperatura muito idênticos e enquadrados na faixa de valores ótimos para o desenvolvimento do pimenteiro (Figura 2.1.). Uma vez que no período inicial ocorre sobretudo crescimento vegetativo da planta, não foram praticamente detetadas alterações entre anos. Outra razão apontada poderá ser a própria variedade utilizada, a Entinas. Visto tratar-se de uma variedade melhorada e muito estável, em condições de crescimento semelhantes, o seu padrão de desenvolvimento será também muito uniforme.

Após plantação registou-se um crescimento normal das plantas, com os maiores crescimentos a serem observados em início de julho. Nesse mesmo período, foram registadas as primeiras flores coincidindo o máximo de floração com os últimos 10 dias do mesmo mês. Os primeiros frutos vingados surgiram pela terceira semana de julho e o máximo a ser registado um mês mais tarde. Os primeiros frutos adultos apareceram no início de agosto estando as plantas em plena produção a partir de finais de agosto (Figura 2.3).

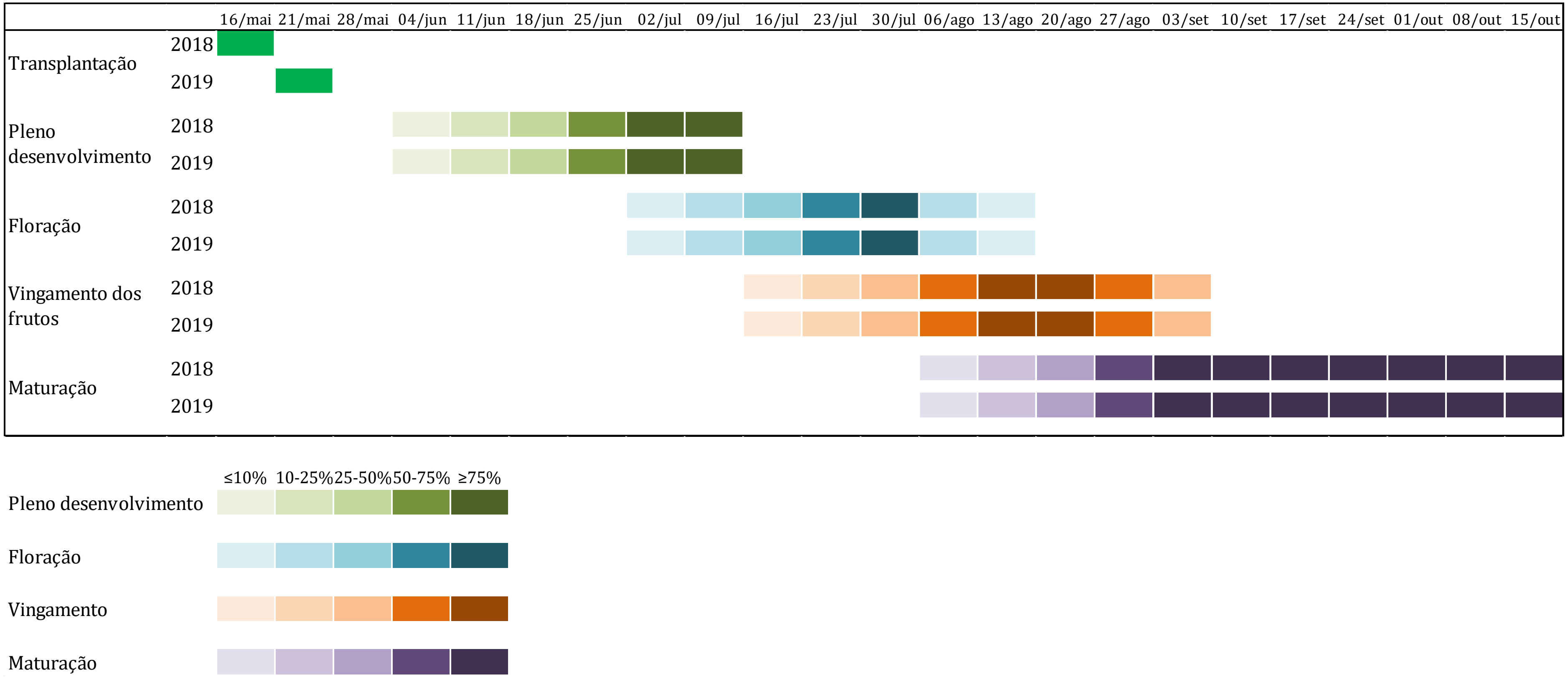

Figura 2.3. Ocorrência dos estados fenológicos de pimenteiro, *Capsicum annuum* L., variedade Entinas, em Coimbra, nos anos de 2018 e 2019. Escala de observação (≤10%; 10-25%; 25-50%; 50-75% e ≥75%) e estados: pleno desenvolvimento, floração, vingamento e maturação.

Nas condições agroecológicas em que decorreu o ensaio, em Bencanta (Coimbra), e para os anos de 2018 e 2019, desde a instalação das plântulas no campo até à última colheita decorreram cerca de 150 dias, 148 dias no ciclo cultural de 2018 e 154 no ciclo cultural de 2019. Na Figura 2.4 faz-se uma representação esquemática dos principais resultados obtidos relativamente ao ciclo de desenvolvimento do pimenteiro.

Figura 2.4. Esquematização da sequência dos estados fenológicos e ciclo cultural do pimenteiro, *Capsicum annuum* L., variedade Entinas, em Coimbra, nos anos de 2018 e 2019.

2.3.2 Produção

Na Figura 2.5 apresentam-se os resultados da produção de pimentos, variedade Entinas, em modo de produção biológico na região de Coimbra. A primeira colheita ocorreu a 19 de agosto, tendo a produção permanecido constante até à colheita de 13 de setembro (Figura 2.5. A), o pico de produção foi registado a 26 de setembro, cerca de um mês e uma semana após o período em que foi observado o número máximo de plantas em plena floração (Figura 2.3.).

Observou-se, também, que os frutos mais pesados foram colhidos na primeira época de colheita, isto é, a 19 de agosto com o peso médio de 163g, e os mais leves a 13 de setembro, em que os pimentos rondavam as 120g. Nas colheitas de 2 e 26 de setembro o peso foi semelhante (142g) enquanto que em outubro pesavam em média 151g (Figura 2.5.B).

Ao reportar a produção por hectare, verifica-se que em cada uma das colheitas foram sempre recolhidos mais do que 4 toneladas/hectare, com valores de cerca do dobro na colheita de 26 de setembro (Figura 2.5. C). Também a produção acumulada por hectare teve crescimentos acentuados ao longo dos diferentes períodos de colheita, atingindo as 28 toneladas por hectare no total das colheitas (Figura 2.5.D).

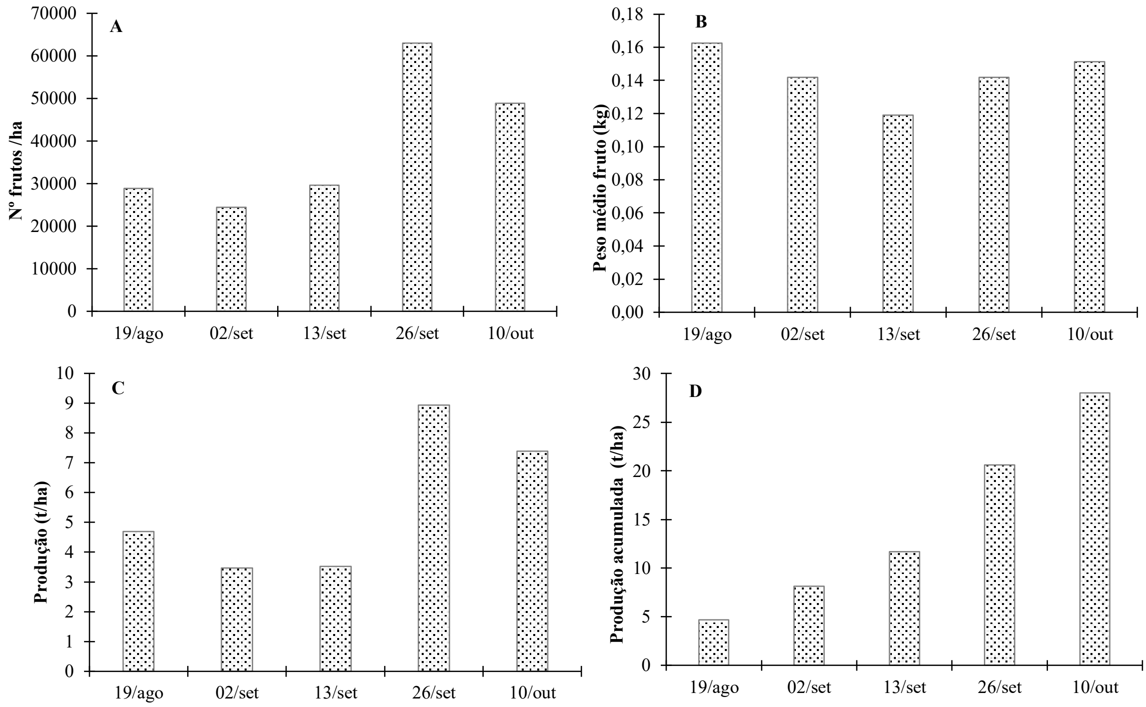

Figura 2.5. Número de frutos recolhidos nas diferentes datas de colheita (A), peso médio do fruto (B), produção por data de colheita (C) e produção acumulada ao longo do ciclo cultural (D) e pimento, *Capsicum annuum* L., variedade Entinas, em modo de produção biológico, Coimbra, 2019.

2.4. Conclusão

Os resultados indicam que o pimenteiro, variedade Entinas, produzido em modo de produção biológico, ao ar livre, na região de Coimbra, apresenta um desenvolvimento normal ao longo da época de produção, com os dados da fenologia da cultura a confirmarem as observações empíricas dos produtores locais.

Constata-se também que a produção de pimento verde biológico apresenta uma produtividade muito aceitável, isto é, cerca de 28 toneladas por hectare, valores que quando comparados com os dados recentes publicados pela FAO (FAOSTAT, 2020) para a produtividade de pimento no mundo, com uma média de 18,5 t/ha, são superiores em cerca de 50%, ainda que fiquem muito aquém dos registados na União Europeia, que ronda as 41,2 t/ha (FAOSTAT, 2020). No entanto é de referir que os dados mencionados englobam toda a produção de pimento, quer o produzido ao ar livre quer em ambiente protegido, e modos de produção biológico e convencional, apesar de ser comummente aceite que o modo de produção biológico apresenta produtividades inferiores.

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Capítulo 3

Elemental Composition and Some Nutritional Parameters of Sweet Pepper from Organic and Conventional Agriculture

Capítulo 3

Referência - Capítulo adaptado da publicação:

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Abstract

The increasing demand of organic agriculture (OA) is based on the consumer's belief that organic agricultural products are healthier, tastier and more nutritious. The effect of OA and conventional agriculture (CA) methods on the elemental compositions of green and red sweet peppers were studied. The highest concentrations of Ca, Cu, K and P occur in peppers from OA in both states of ripeness, with emphasis on Ca and K contents. Furthermore, the principal component analysis (PCA), points out to a clear separation, regarding concentrations, between peppers from OA and CA. The average fruit weight is higher in OA, 141 g *versus* 112 g in CA. Regarding productivity, CA reaches a value of 30.1 t/ha, 7% higher than the value observed for OA, i.e., 28 t/ha. Peppers from CA, exhibited greater protein content than those which originated from OA, regardless of the ripening stage, but not more ashes. Regarding nutritional ratios, the ripening stage and the production mode, can be important for an adequate choice regarding a more balanced Ca/P ratio, and the studied variety contained high Ca values ranging between 1 009 and 1 930 $mg.kg^{-1}$. The PCA analysis also revealed that Mn and Fe are inversely correlated, confirming the importance of the Mn/Fe ratio evaluation in nutritional studies.

Keywords: nutritional characteristics; production mode; ripening stages; sweet peppers.

Capítulo 3

3.1. Introduction

Current agricultural systems have been mainly focused on yield rather than on balanced energy input/output ratios or on the sustainability of ecosystems and food production. It is well known that the intensive use of fertilizers, machinery and the agricultural practices as a whole, contribute to large greenhouse gas (GHGs) emissions (Smith et al., 2019). Thus, farmers are facing a dilemma between pursuing their activities and maintaining or increasing their income, or adapting to climate change (CC) scenarios and reducing GHGs emissions.

Whatever the strategies for feeding the world in a more sustainable way, we currently assist a huge expansion of organic agriculture (OA) mainly based on an environmental awareness of the consumers and concerns with their food safety despite the criticism of some authors. According to the latest survey on OA, the organic farmland increased substantially, as well as the number of organic producers and organic retail sales (Willer & Lernoud, 2019). In fact, although organic production currently accounts for only 1.4% of global agricultural land with approximately 70 million ha in 2017, it is responsible for a global organic market that has reached 97 billion US dollars in the same year (approx. 90 billion euros), involving almost three million producers worldwide.

The critics have long argued that organic agriculture is inefficient, requiring more land to yield the same amount of food (Smith et al., 2019). However, when organic and conventional yields were compared using a new meta-dataset three times larger than previously used (115 studies containing 1 071 observations, involving 38 countries and 52 crop species over a span of 35 years) it was observed that organic yields are only 19.2% lower than conventional yields, a smaller yield gap than previous estimates (Ponisio et al., 2015). The same authors did not find significant differences in yields for leguminous and non-leguminous crops, nor for perennials and annuals, nor between the yield gaps for studies conducted in developed versus developing countries (Ponisio et al., 2015).

One of the most important functions of different agricultural production systems is to provide almost all essential mineral and organic nutrients to humans (Wang et al, 2008). Additionally, there seems to be a widespread perception among consumers that organic farming results in products of higher nutritional quality (Williams, 2002). It was concluded that organic products contained significantly more vitamin C, Fe, Mg and P, and significantly less nitrates and heavy metals when present, than products from conventional agriculture (Worthington, 2004). Most well-designed studies comparing nutrient density (milligrams of a given nutrient per kilogram of food) in organically and conventionally produced fruits and vegetables, show simple to moderately higher concentrations of nutrients in organic products (Benbrook, 2009).

Furthermore the evaluation of the mineral content of several edible products or even food supplements is paramount, since it allow us to monitor the enrichment or the poorness of essential elements to human nutrition and metabolism, or even detect possible contamination by heavy metals such as Cd, Pb, and Hg, among others (Reboredo et al., 2018; Reboredo et al.,2019; Reboredo et al, 2020). In this context it is also useful to perform an acute characterization of the soil since potential redox, cation exchange capacity and particularly pH influences decisively affect the solubility and/or availability of the nutrients and the uptake by plants (Reboredo et al., 1984; Pelica et al., 2018; Carrondo et al., 1984), beyond intrinsic aspects of the plants—that is, mainly related to species, varieties, development status and metabolic requirements, as well as to possible abiotic stresses that may occur (Reboredo et al., 2001; Reboredo et al., 2012).

Organic farming in Portugal started flourishing in the nineties, as the land and number of organic farmers had average annual growth rates above 20% and 40%, respectively (Matos et al., 2016). In 2017, the Portuguese mainland area of organic production occupied 252,812 ha and was mainly dedicated to pastures and cattle fodder to feed livestock (72%), while approximately 26% of the area was dedicated to the production of foodstuff for direct consumption or for processing (DGADR, 2019), and horticulture was included with only 1.2% of the area. Thus, the reduced offering of several highly appreciated products such as pepper, courgette, beans and lettuce for example, limits its sales growth in natural and gourmet food stores.

Pepper (*Capsicum annuum* L.) is one of the most important vegetables being produced and consumed in several countries worldwide. Pepper is cultivated in Portugal as a spring-summer crop in the open air. The aim of the present study is to evaluate the differences, regarding the elemental composition, and other nutritional characteristics, of two different cultures of sweet pepper (*Capsicum annuum* L.) using organic and conventional agricultural approaches and taking into account two different ripening stages. This information will contribute to increase the knowledge of the effect of the organic production method on the pepper´s nutritional value.

3.2. Materials and Methods

3.2.1. Plant Cultivation

Two producers of sweet peppers (*Capsicum annuum* L.), located in the central region of Portugal (Coimbra) were selected with the following coordinates: Organic agriculture—40°13′4.71″ N (latitude); 8°26′58.28″ W (longitude). Conventional agriculture—40°13′14.00″ N (latitude); 8°28′29.22″ W (longitude). The organic producer, followed the European Commission Guidelines (Council Regulation (EC) nº 834/2007 of 28 June 2007) whereas the other producer, had no limitations on the use of fertilizers and pesticides.

Sweet pepper seedlings, from the Entinas variety, were put in the soil in May 2018 (Table 3.1), and grew under open field conditions until September, i.e., it took approximately four months between planting and harvesting sweet peppers. Each field had 800 m^2 and the density of plantation was 22 222 plants/ha corresponding to a distance between the lines of 0.75 cm and between plants of 0.60 cm.

Table 3.1. Phenological development stages of sweet pepper from organic (OA) and conventional (CA) agriculture (% of plants).

Stage Development	Production mode	16/may	21/may	28/may	04/jun	11/jun	18/jun	25/jun	02/jul	09/jul	16/jul	23/jul	30/jul	06/aug	13/aug	20/aug	27/aug	03/sept	10/sept	17/sept	24/sept
Transplanting	OA																				
	CA																				
Leaf Development	OA				≤10%	10-25%	25-50%	50-75%	≥75%	≥75%											
	CA				≤10%	10-25%	50-75%	50-75%	≥75%	≥75%											
Flowering	OA								≤10%	10-25%	25-50%	50-75%	≥75%	25-50%	10-25%						
	CA								≤10%	25-50%	25-50%	50-75%	≥75%	25-50%	10-25%						
Development of Fruit	OA										≤10%	10-25%	25-50%	50-75%	≥75%	≥75%	50-75%	25-50%			
	CA										≤10%	25-50%	25-50%	50-75%	≥75%	≥75%	50-75%	25-50%			
Ripening of Fruit	OA													≤10%	10-25%	25-50%	50-75%	≥75%	≥75%	≥75%	≥75%
	CA													≤10%	25-50%	25-50%	50-75%	≥75%	≥75%	≥75%	≥75%

The distance between experimental fields was approximately 950 m and the meteorological conditions registered in the area by the Agrarian School of the Polytechnic Institute of Coimbra (where the fields are located) are described as follows: temperature (°C) - maximum (22.3; 24.2; 26.1; 32.0 and 31.5), average (16.4; 19.0; 20.9; 22.7 and 21.4) and minimum (11.0; 15.1; 16.8; 15.7 and 15.0); evapotranspiration (mm) - (98.7; 92.5; 109.6; 123.6 and 95.0); relative air humidity (%) - (78.8; 84.6; 82.2; 74.4 and 77.4); rainfall (mm) - (37.6; 98.8; 7.8; 0.0 and 1.2). The values are presented in a sequential manner, i.e., the first one is from May, the second from June, the third from July, the fourth from August and finally, the fifth from September.

Soil characterization can be indicated as follows: OA (pH 6.4; organic matter 1.5%; extractable P - mg P_2O_5 kg^{-1}, 105; extractable K - mg K_2O kg^{-1}, 156); CA (pH 6.0; organic matter 1.8%; extractable P - mg P_2O_5 kg^{-1}, 181; extractable K - mg K_2O kg^{-1}, 134).

In both fields, a drip irrigation system was installed and the nutritional requirements were supplied by horse manure, in OA, and by chemical fertilizers, in CA. The water for irrigation came from the Mondego River (pH 7.34; EC 0.10 mS/cm^{-1}; SAR 0.8 meq L^{-1}). The drip irrigation system had a flow rate of 2.66 L/s per emitter for approximately 20 min with irrigation intervals between 2 or 3 days, depending on evapotranspiration conditions and precipitation occurrence.

Regarding fertilization of CA, it was made with chemical fertilizers: before planting (700 kg/ha) with: 7% N, 14% P_2O_5, 14% K_2O, 3% CaO, 2% MgO, 9% SO_3 and 0.02% B; and at flowering, late July, (300 kg/ha) with 27% N and 4% CaO. In OA, horse manure was incorporated into the soil before planting (29.6 t/ha) with 5.8 g of N/kg^{-1}, 2.8 g of P_2O_5/kg^{-1} and 5.3 g of K_2O/kg^{-1}. The weeds control was performed with herbicide (CA) and with mechanical and manual methods at OA. In CA, two weeks before crop planting a selective weed herbicide was applied (glyphosate, 4–5 L/ha).

The phenological development stages of sweet pepper from OA and CA during 2018, can be observed in Table 3.1. It is clear that peppers from CA initiate in advance the leaf development, the flowering and the development of the fruit, expressed as percentage of plants where these stages were observed, which is probably related to the use of fertilizers and particular characteristics of the soils, despite being transplanted into the soil five days after organic plants.

3.2.2. Sample Collection

In the middle of September 2018, from each producer (organic and conventional) sweet peppers from each producer were harvested on the same day at two different maturation stages, corresponding to green and red colours. Four independent batches with approximately 2 kg each (two for each production mode), were collected. Each batch containing 15 sweet peppers was then divided into five groups of three units. From each group one pepper was removed to perform moisture and elemental determination.

A total of 60 peppers were used for analysis. Forty of them were washed, cleaned, air-dried, and further stored under refrigeration (~4 °C), until analysis (protein, TA, TSS-°Brix, fiber, ash). The determination of firmness, was made prior to storing samples in the refrigerator. The remaining 20, previously washed and cleaned, were cut in slices and the edible part was separated from non-edible (seeds). Samples were weighed (fresh weight) and dried at 65 °C until constant weight. After the determination of dry matter and moisture, powder reduced samples in an agate mortar were used for XRF analysis.

Productivity (t/ha) was derived from 30 selected plants along the harvesting phase. Approximately 260 peppers from OA and 370 from CA were collected and weighed after careful rinsing with distilled water.

3.2.3. Nutritional Composition

The methodologies of the Association of Official Analytical Chemicals (AOAC, 1997) were used to determine chemical properties of the dried pepper samples, namely, crude fiber (method 930.10), ash (method 930.05) and crude protein (method 978.04). Additional detailed information is found in (Barroca et al., 2020).

Regarding the firmness the maximum penetration force (N) was evaluated with a HD plus texture analyzer (Stable Microsystems, Godalming, UK). The evaluation was made by penetration with a 2 mm cylinder probe, with a 5.0 kg (50 N) charge cell, and with a test speed of 1.0 mm/s and 10 mm length (IP, 2019).

The titratable acidity (TA) was determined by titrimetric analysis, with a NaOH solution (0.10 mol/L). Approximately, 10 g of each sample (previously ground) were mixed with 50 mL of water and put on heating under reflux for 30 min. Then, the resultant solution was transferred to a glass balloon of 100 mL and, after filtration, a precise volume (20 mL) was transferred to a beaker with a stirrer. Then, the pH of the solution was monitored continuously in order to obtain the titration curve. The values were expressed on mg citric acid/100 g fresh weight (Mitcham et al., 1996).

The pH values were evaluated using a Crison-Micro pH 2002 (Crison, Barcelona, Spain) potentiometer. The solution obtained for the acidity determination (after filtration) was also used to measure total soluble solids (TSS) contents (°Brix), at 20 °C, in an ATAGO refractometer (Saitama, Japan) (Mitcham et al., 1996).

3.2.4. XRF Preparation and Analysis

The fine powder of dried pepper samples was pressed for 2 min under 10 tons in order to make a cylindrical pellet with a diameter of 20.0 ± 0.5 mm and a thickness of 1.0 ± 0.5 mm. This pellet was then glued onto a mylar sheet in a plastic frame and placed directly onto the X-ray beam for analysis.

All of the elemental quantifications were obtained using the micro-energy dispersive X-ray fluorescence (μ-EDXRF) system (M4 TornadoTM, Bruker, Germany). This commercial spectrometer consists of an air-cooled micro-focus side window Rh-anode X-ray tube, powered by a low-power HV generator. The system features a poly-capillary X-ray optic, which allows a beam spot size of around 22 μm for Rh Kα. In all of the measurements, the X-ray generator was operated at 50 kV and 100 μA without the use of filters, in order to enhance the ionization of low-Z elements without compromising the peak-to-background ratio for medium-Z elements such as Mn, Fe, Cu and Zn (Cardoso et al., 2018). Detection of the fluorescence radiation is performed by an energy-dispersive silicon drift detector, XFlashTM, with a 30 mm2 sensitive area and an energy resolution of 142 eV for Mn Ka.

In order to obtain an average spectrum that is representative of the whole pellet, elemental maps were acquired with a pixel spacing of 15 μm and a measuring time of 6 ms per pixel. Quantification of the spectra of the obtained maps was performed with the fundamental parameters method of the built-in ESPRIT software and the recovery rate was checked against a set of standard reference materials—orchard leaves (NBS 1571), poplar leaves (GBW 07604) and tea leaves (GBW 07605).

The achieved detection limits with this setup can be seen in reference (Gallardo et al., 2016) and are around 5, 4, 55, 35, 4 and 15 μg/g, for S, Cl, K, Ca, Mn and Fe, respectively. For Cu and Zn, the detection limit is 2 μg/g. The Na and Mg levels, were not assessed, since elements with low atomic number (<13) were not quantified by μ-EDXRF, unless present in large concentrations. Thus, they are referred as below the detection limits.

3.2.5. Statistical Analysis and Control Assurance

Statistical analysis of the data was performed with the SPSS Statistics 18 program, through an analysis of variance (ANOVA) and the F-test. A value of $P \leq 0.05$ was considered to be significant. All analyses were made in quintuplicate. Analytical accuracy was verified using replicate determinations and Standard Reference Materials, as referred above, with percentages of recovery ranging between 92% and 99%. Principal component analysis was applied to observe any possible clusters within analyzed sweet peppers from OA and CA in two different ripening stages (green and red) with the STATISTICA (data analysis software system), version 12.

3.3 Results

3.3.1. Nutritional Characterization of Sweet Peppers

The elemental concentrations of sweet peppers, produced in organic agriculture (OA) and conventional agriculture (CA), and harvested in two gradual stages of ripeness (green and red) are presented in Table 1. The concentrations of macronutrients in sweet peppers in both modes of production and states of ripeness exhibits the following descending order: potassium (K) > phosphorus (P) > calcium (Ca) > and sulphur (S). Regarding the micronutrients, the descending order is: chlorine (Cl) > iron (Fe) > zinc (Zn) > manganese (Mn) > copper (Cu)—Table 3.2.

Table 3.2. Average concentrations of sweet peppers originated from organic and conventional agriculture.

Elements	Green peppers		Red peppers	
	Organic	Conventional	Organic	Conventional
K	4,02±0,299a	3,55±0,290ab	3,65±0,276ab	3,27±0,345b
Ca	1930±144,8a	1009±97,10b	1314±365,8b	1041±203,3b
P	1974±394,7a	1528±208,6a	2044±281,38a	1704±313,2a
Fe	84,1±10,4a	64,7±5,4b	68,0±13,9ab	73,6±8,5ab
Zn	19,2±2,27b	25,0±2,43a	15,7±1,87b	18,6±1,82b
Mn	7,37±2,07b	21,3±4,51a	6,49±1,92b	22,5±11,5a
S	1644±329,9a	1518±216,3a	1232±162.2a	1566±204,5a
Cu	11,8±4,21a	11,6±2,21a	9,32±0,99a	7,94±4,33a
Cl	3892±630,9a	3250±578,5a	2862±959,2a	2984±437,1a
Ca/P	0.98	0.66	0.64	0.61
Mn/Fe	0.087	0.329	0.095	0.306
Fe/Cu	7.13	5.58	7.30	9.27
Zn/Cu	1.63	2.16	1.68	2.34

The mean values are expressed in % (K) and mg kg^{-1}, on a dry weight basis (remaining elements) ± standard deviation; n = 5; the mean values in the same row, followed by common letters, are not significantly different at 0.05 significance level.

The K levels range between 3,27% and 4,02%, P between 1 528 mg kg^{-1} and 2 044 mg kg^{-1}, Ca between 1 009 mg kg^{-1} and 1 930 mg kg^{-1}, and finally S between 1 232 mg kg^{-1} and 1 644 mg kg^{-1}. In what regard to micronutrients, Cl had the highest concentrations ranging from 2 862 mg.kg^{-1} to 3 892 mg kg−1, Fe from 64,7 mg kg−1 to 84,1 mg kg^{-1}, Cu from 7,94 mg kg^{-1} to 11,8 mg kg^{-1}, Zn from 15,7 to 25,0 mg kg^{-1}, and finally Mn from 6,49 mg kg^{-1} to 22,5 mg kg^{-1} (Table 3.2).

In the case of K, P, Ca and Cu, the concentrations in peppers from organic agriculture (OA) were higher than those observed in peppers from conventional agriculture (CA), regardless of whether they were red or green. In fact, in the case of green peppers (OA) the concentrations of K, P, Ca and Cu were 1.29, 1.13, 1.02 and 1.91 times higher, respectively, than the equivalents from CA (Table 3.1). However, it should be noted that with the exception of Ca, the mean values were not significantly different ($P \leq 0.05$), so the differences observed are not relevant, fitting the variability of a given population.

In the case of red peppers (OA), the concentrations of K, P, Ca and Cu were 1.12, 1.20, 1.17 and 1.26, times higher, respectively, than the equivalents observed for CA. Also, in this case, the mean values were not significantly different ($P \leq 0.05$).

In relation to Mn and Zn, the highest concentrations were observed in peppers from CA, both green and red, compared to those derived from OA. In the case of green peppers (GP), there are significant differences between the averages observed in both modes of production and for both elements, whereas for red peppers (RP) there are also significant differences between the modes of production, but only for Mn.

Furthermore, Mn was the element where the greatest differences between the different cultivation modes were noted—for example, in green peppers from CA, the average concentration was approximately 3.0 times higher than that observed in peppers from OA. A similar situation occurred for red peppers (CA) that presented concentrations about 3.5 times higher than those of (OA).

In relation to Cl, Fe and S, there are differences that vary according to the ripening stage and the type of production. The concentrations of Cl, Fe and S in GP are higher in OA, the opposite is true for RP derived from CA. In a broader analysis, regardless of the type of ripening stage and the type of culture, there are no significant differences ($P \leq 0.05$) with respect to the observed concentrations of Cl, Cu, K, P and S. Only in the case of Ca in organic GP (1 930 mg kg^{-1}) was a statistically significant difference was observed. A similar situation occurs for Zn, as well as for GP but from CA (Table 3.2).

Regarding the Ca/P ratio, it was observed that for organic GP this value was 0.98, while for conventional GP, this value was 0.66. In the case of red peppers, the Ca/P ratio was very close—0.64 vs. 0.61 for OA and CA, respectively. The Mn/Fe and Zn/Cu ratios are higher in CA, while the Fe/Cu ratio is similar in OA regardless of the ripening state, whereas in CA this ratio increases from 5.6 (GP) to 9.3 (RP) (Table 3.2).

Peppers from CA, exhibited more protein that those originated from OA, regardless of the ripening stage. Conversely, the ash content of RP and GP from OA is close, ranging from 5.87% to 6.38%, respectively, but higher than the values observed in CA, which range from 4.98% to 5.03%, for green and red, respectively. No significant differences were observed in the fiber content of GP from OA and CA ($P \leq 0.05$); the same occurred with RP (Table 3.3).

Table 3.3. Nutritional characterization of sweet peppers from organic and conventional agriculture.

	Green peppers		Red peppers	
	OA	**CA**	**OA**	**CA**
Fruit weight	159±37,7a	127±16,1ab	123±13,8ab	97.4±18,9b
Moisture*	93.9±1,30a	92.5±0,60a	91.8±0,75a	90.3±0,43b
Protein*	10.3±0,62b	11.7±0,56a	9.17±0,49b	11.9±0,42a
Ash*	6.38±0,58a	4.98±0,32b	5.87±0,28a	5.03±0,27b
Fiber*	11.4±0,93a	11.6±0,74a	9.23±1,00b	10.3±1,06ab
Firmness**	12.1±2.3a	7.9±0.9b	9.2±1.4ab	7.9±1.2b
pH**	6.2±0.1a	6.0±0.5a	5.0±0.1b	5.2±0.1b
TSS(ºBrix)**	3.8±0.3c	4.4±0.5c	5.8±0.7b	7.6±0.5a
TA**	63±4.0b	67±9.0b	162±16.0a	171±18.0a
	Organic Agriculture		**Conventional Agriculture**	
Productivity	28.0		30.1	

(*) The mean values are expressed in percentage (%) ± standard deviation; fruit average weight (g) ± standard deviation; n = 5; productivity (t/ha) was derived from 30 selected plants along the harvesting phase. (**) Firmeness, pH, total soluble solids (TSS) and titratable acidity (TA) expressed as mg citric acid/100 g fresh weight, from Guilherme et al, 2020. The mean values in the same row, followed by common letters, are not significantly different at 0.05 significance level.

The average fruit weight is higher in OA, with a value of 141 g per unit versus 112 g in CA. However, in terms of productivity, CA products a value of 30.1 t/ha slightly higher than the value observed for OA, i.e., 28 t/ha. In both methods (OA and CA), during the ripening process fruit weight decreases by approximately 23%.

3.3.2. Principal Component Analysis (PCA)

In order to jointly evaluate the influence of the production method and the state of ripeness, an analysis in main components (PCA) was carried out, using the data of OA and CA in two different ripening stages. Eight attributes (elements) were used, namely: P, Ca, Cu, Fe, Mn, K, Zn and S.

The first two main components explained the cumulative percentage of 65.25%:39.19% for the first component and 26.06% for the second. Only these components were significant, since they are those with an own value > 1. Thus, as they have an own value > 1, the first two components were defined as main components: the first had an own value of 3.1 and the second an own value of 2.1 (Table 3.4).

Table 3.4 Principal component analysis (PCA). Percentage of variance for each component (initial eigenvalues) and correlation coefficients (component matrix) of each variable with component 1 (PC1) and 2 (PC2).

Principal Component	Eigenvalue	Total Variance (%)	Cumulative Eigenvalue	Cumulative (%)
1	3,13	39,19	3,13	39,19
2	2,08	26,06	5,22	65,24
3	0,89	11,09	6,11	76,33
4	0,63	7,93	6,74	84,26
5	0,44	5,51	7,18	89,76
6	0,33	4,17	7,52	93,94
7	0,25	3,17	7,77	97,11
8	0,23	2,89	8,00	100,00

In order to understand the relative importance of each attribute in relation to each of the first two main components, the correlation coefficients between the attributes (original parameters) and the main components were determined (Table 3.5). The results of the 1st principal component (PC1), are explained, by P, Ca, Fe and K (with negative correlation values) and Mn (with positive correlation values), whereas the results of the 2nd principal component (PC2) are explained by Zn and S (with positive correlation values).

Table 3.5. Correlation coefficients between attributes (initial variables) and PC1 and PC2.

	Components	
Attribute	PC1	PC2
P	-0,68*	-0,02
Ca	-0,82**	0,09
Cu	-0,37	0,55
Fe	-0,75**	0,32
Mn	0,62*	0,57
K	-0,86**	-0,19
Zn	0,26	0,83**
S	-0,29	0,79**

In Figure 3.1 it can be observed that the projection of the samples in the main plane, constituted by the first two components, is associated with the approximate projection of the attributes in the main plane.

The implementation of PCA, allow us to take the following results:

1—The clear separation, with regard to concentrations, between peppers from OA and CA.

2—Organic peppers tend to have higher concentrations of Ca, Fe, K and P and less Mn.

3—In general, GP from both production methods have higher concentrations of S and Zn.

4—In general terms, conventionally grown GP have a higher concentration of Mn and Zn than all the others.

5—The Cu shows no significant correlation with any of the axes.

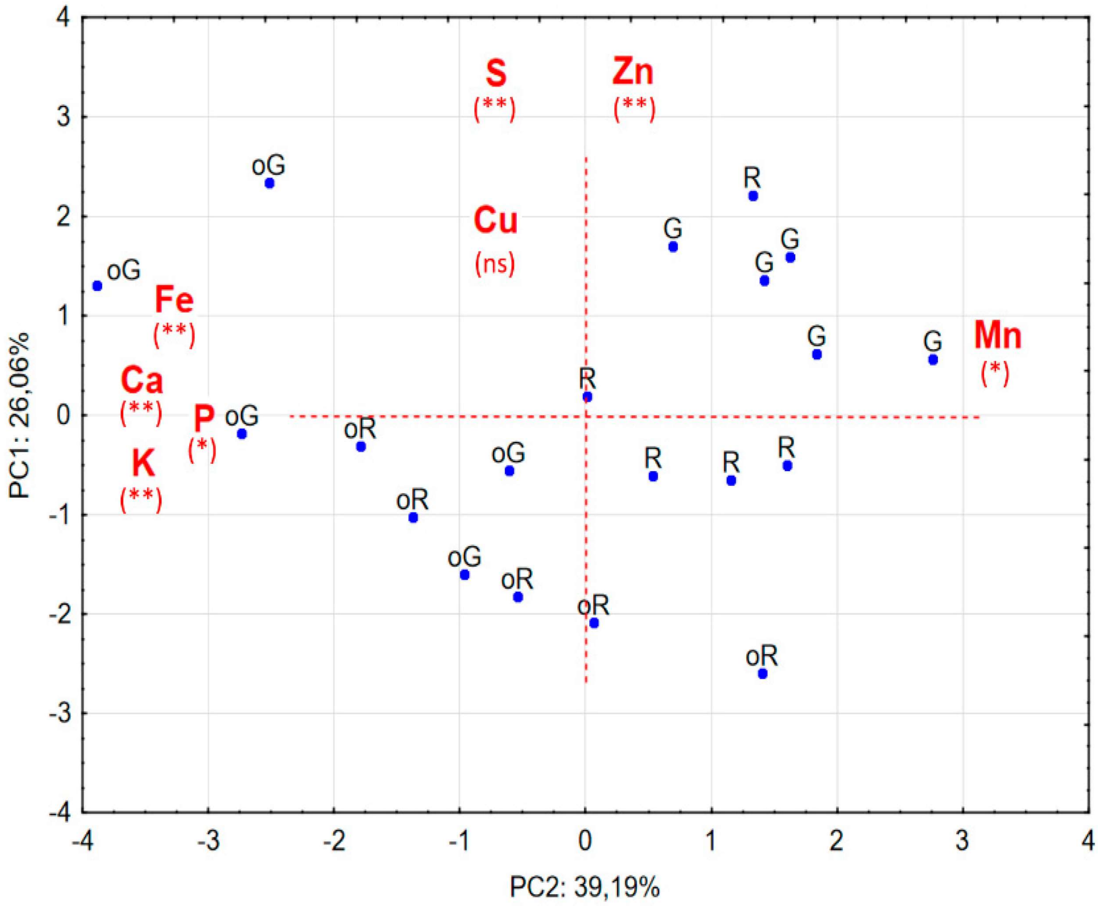

Figure 3.1. Analysis of main components: PC1 vs. PC2 projection of samples (n = 5). The most important variables for the definition of the two components are shown in each axis, indicating the direction in which each element grows. Five samples of each of the peppers, are presented in the projection: peppers obtained in conventional production mode, red (R) and green (G) and peppers obtained in organic production mode, oR and oG. * Moderately significant correlation values between the element and the PC; ** strongly significant correlation values between the element and the PC; ns = non-significant correlation between PCs.

3.4. Discussion

Potassium is the most abundant element followed by P and Ca. This distribution is similar to that observed in two Spanish varieties of *C. annuum* (Bernardo et al., 2008). Regarding micronutrient concentrations (Bernardo et al., 2008), the following order is observed: Fe > Mn > Zn > Cu. In the current study Zn ranks second while Mn is in the third position. The average concentrations of K detected by us are clearly higher than those found in peppers collected in areas as distinct as the Canary Islands(Spain) (Rubio et al., 2002), Valencia(Spain) (Ribes-Moya et al., 2014), Ethiopia (Esayas et al., 2011), or South Korea (Kim et al., 2019).

The elementary composition of GP and RP from the Canary Islands (Rubio et al., 2002), revealed a maximum K concentration of 250 mg 100−1 for RP and 199 mg 100−1 for GP. In the case of Ca the maximum value for RP was 33.2 mg 100−1 and 23.4 mg 100−1 for GP, while in the case of P, it was 40.0 mg 100−1 (RP) and 50.3 mg 100−1 (GP). The above-mentioned concentrations were clearly lower than those observed in the present study.

Other studies, however, report concentrations more closer to with those detected in the current work. When studying 17 varieties of peppers (Ribes-Moya et al., 2014), it was observed that the average K concentration in GP was 2898 mg 100−1 versus 2155 mg 100−1 for RP. The same authors point out the average concentrations of P of 283 mg 100−1 for GP and 225 mg 100−1 for RP (Ribes-Moya et al., 2014).

It was found that K concentrations are higher in GP, regardless of agricultural practices, while P concentrations are higher in RP, which partially agrees with the data (Ribes-Moya et al., 2014). The analysis of 12 varieties of RP grown in two different regions of South Korea (Kim et al., 2019), showed that the average levels of Fe in the Imsil (IS) and Youngyang (YY) regions were 6.1 mg 100^{-1} and 6.6 mg 100^{-1} respectively. Regarding Ca, the same authors point out the mean values of 114 mg 100^{-1} in IS and 104 mg 100^{-1} in YY, while for K the mean concentrations were 2 821 mg 100^{-1} (IS) vs. 4125 mg 100^{-1} (YY), levels quite concordant with those detected in the current work.

The variability of the data is also documented in an extensive study, regarding vitamins A and C, folate, and capsaicin concentrations of different pepper types, emphasizing the huge variations within species, varieties, color, and geographic locations (Kantar et al., 2016). Other details can also explain the variability. While in some works (Bernardo et al., 2008; Kim et al., 2019), the analysis is carried out with dehydrated samples (in our case reduced to powder) in others (Rubio et al., 2002; Ribes-Moya et al, 2014), it is not clear whether the samples for analysis are weighed without dehydration, which occurs a posteriori when determining the elemental composition.

Additionally, the effect of fruit maturation on quantitative changes in elemental composition and organic compounds must also be taken into account. For example, in *Capsicum annuum*, *Capsicum frutescens*, and *Capsicum chinese*, the concentration of carotenoids, flavonoids, total soluble reducing equivalents, phenolic acids, ascorbic acid, and antioxidant activity generally increased with maturity (Howard et al., 2000), although in two common Spanish *Capsicum* varieties, none influence on elemental concentration was observed as a function of the ripening stage (Bernardo et al., 2008).

The protein content of two Spanish *Capsicum* varieties ranged between 11.37% and 12.02% regardless of the ripening stage (Bernardo et al., 2008), while the variation within three *Capsicum* varieties from Ethiopia was between 8.7% and 11.8% (Esayas et al., 2011). The same authors observed that the ash content varied between 4.27% and 4.68% (Bernardo et al., 2008) and between 5.3% and 7.3% (Esayas et al., 2011). The mentioned values are in good agreement with our values, although other studies reported much higher values of both protein and ashes (Kim et al., 2019).

The application of N, P, K and S fertilizers increased crop yield and protein concentration in cereals and pulses, and concentration of essential amino acids and vitamins in vegetables. However, excessive fertilizer use, especially N fertilizer, can result in undesirable changes such as increases in nitrate, titratable acidity and acid to sugar ratio, while decreasing the concentration of vitamin C, soluble sugar, soluble solids, and Mg and Ca in some crops (Wang et al., 2008), which agrees with our results since fiber and protein contents are higher in CA peppers.

Also, titratable acidity (TA) is similar in RP, regardless of the type of production, thus indicating that TA increases during the ripening process in parallel with a decrease of pH. A similar situation occurs for total soluble solids (TSS). In this context, it does not seems to us that an excessive fertilizer use had occurred (Wang et al., 2008), and the differences observed are mainly related to the variety itself since the agro-environmental factors are similar, except the fertilization process, although other authors point out to an inverse relationship between TA and pH—during the ripening of cactus Cereus peruvianus fruit, the TA decreased and the pH increased (Ninio et al., 2003), which also occurred during the ripening of blackberry fruit (Tosun et al.,2008) they make the assumption more cristal-clear.

Organic foods (vegetables, legumes and fruit) were found to have a 5.7% higher content of vitamins and minerals than their conventionally grown counterparts with emphasis on P levels (Hunter et al., 2003). These results were explained by the hypothesis of accelerated growth, as a result of conventional agricultural methods, that down-regulates the synthesis of carbon-containing metabolites, such as ascorbic acid (Hunter et al., 2003). In our case both P and ash levels are higher in OA peppers.

The average weight of each fruit, regardless of the ripening stage and the production mode, varied between approximately 100 g and 150 g, a very low weight when compared with the range of 350 g and 500 g, observed in two different varieties (Bernardo et al., 2008), emphasizing the great variability among varieties, beyond the production mode and substrata characteristics. Furthermore, our production around 30 t/ha is greater than the world average production of chilies and peppers (*Capsicum* spp.) in 2018, which is 18.5 t/ha but below the average of EU28 in the same year, which is 41.2 t/ha. Despite China having an average of 23.6 t/ha, it is responsible for approximately half of the world production (FAOSTAT, 2020).

Regarding the Ca/P ratio, lower values (0.52 and 0.51) were observed for two varieties of *C. annuum* from Nigeria (Ogunlade, 2012), whereas the Ca/P ratio values in a Spanish *C. annuum* variety decreases during the ripening process, from 1.29 (GP) to 0.61 (RP) (Rubio et al., 2002), *Capsicum annuum* is one of the plant species with the lowest levels of Ca (<8.7 mg/100 g), whereas P is present in vegetables in the range of 16.2–437 mg/100 g (Martinez-Ballesta et al., 2010), thus a prevalence of P over Ca is common.

The Ca/P ratio is important for human nutrition Excessive intake of P per se can be harmful to bones through increased parathyroid secretion, but the adverse effects on bone mass increase, when dietary Ca intake is clearly low (Kemi et al., 2010). In many countries, P intake is abundant, while Ca intake does not meet the recommendations, so it is difficult to achieve an optimal proportion of Ca/P in the diet (Kemi et al., 2010). Thus, the ripening stage and the production mode of peppers, can be important for an adequate choice regarding a more balanced Ca/P ratio.

The negative interaction of metal ions is one of the main dietary factors that causes low bioavailability of these nutrients. These include Na-K, Ca-Mg, Mn-Fe, Fe-Cu, and Zn-Cu, the latter being the most significant in human nutrition due to the negative effect of excess Zn on Cu bioavailability, i.e., when the first metal of each pair is in excess and the other is at the lower limit of requirement (O´Dell, 1989). In our case the Zn/Cu ratios range between 1.63 and 2.34 indicating that a good balance exists between these two elements without compromising Cu availability.

In regard to Mn-Fe interactions, it was observed that Mn inhibited iron absorption both in solutions and in a hamburger meal in human trials, most probably due to similar absorptive pathways (Rossander-Hultén, 1991). In the same experiment with Zn, no inhibitory effect was observed, suggesting different pathways for the absorption of Zn and Fe. In our case the Mn/Fe ratios did not exceed 0.329, indicating that Mn is present in very low amounts in sweet peppers. Furthermore, in the principal component analysis, Mn and Fe are inversely correlated in component 1 (PC1)—one positively (Mn: 0.62) and the other negatively (Fe: −0.75).

Adequate Cu nutritional status is necessary for normal Fe metabolism and red blood cell formation, indicating an interconnection between Cu availability and Fe metabolism in humans

(Collins et al., 2010; LPI, 2017). For example, infants fed a high-iron formula absorbed less Cu than infants fed a low-iron formula, suggesting that high Fe intakes may interfere with Cu absorption (FNB, 2001). The sweet pepper Fe/Cu ratios range between 5.58 and 9.27 indicating a prevalence of Fe over Cu.

One may argue that the search of ideal elemental ratios is not worthwhile because there is a difference between the dietary ratio and the blood (or serum) ratio, the latter more relevant in health and disease (Osredkar & Sustar, 2011). However, the load of a particular element in a certain foodstuff may well give us an indication of balance/imbalances, without losing the focus on the interactions and bioavailability and as recently referred, the utilization of dietary micronutrient ratios in nutritional studies might well be more informative than focusing on a single nutrient (Kelly et al., 2018; Kelly et al., 2019).

For example, a high intake of Fe, especially in combination with high Mn intake, may be related to risk for Parkinson´s disease (Powers et al., 2003), thus, consumers must avoid foodstuffs particularly rich in both elements. The awareness of these interactions, combined with a balanced evaluation of the dietary intake, could lead to more effective strategies to improve micronutrient status (Sandstrom, 2001), although currently, the biofortification of staple foods (Pataco et al., 2017.; Lidon et al., 2018; Mangueze et al., 2018) and the consumption of food supplements (Reboredo et al., 2020) stay ahead, despite the negative effects of Fe supplementation on indices of Zn and Cu status and of Zn supplementation on Fe and Cu status that have been reported (Sandstrom, 2001).

The production of *Capsicum* species is cheap and easy, therefore, integrating a pepper-rich diet in our daily meals can be helpful in alleviating micronutrient deficiency, especially in poor households in developing countries (Olatungi & Afolayan, 2018) thus preventing the appearance of chronic diseases.

3.5. Conclusions

The highest concentrations of Ca, Cu, K and P occur in peppers from OA regardless the state of ripeness. Furthermore, the PCA highlights out to a clear separation, regarding concentrations, between peppers from OA and CA.

In general terms, green peppers from both production methods have higher concentrations of S and Zn, but they are also a good source of Ca and K. In what regard to nutritional ratios it is important to emphasize that, the ripening stage and the production mode, can be important for an adequate choice regarding a more balanced Ca/P ratio. In the case the Mn/Fe, Mn is present in very low amounts and does not seem to influence the Fe absorption. The adequate selection of micronutrient-rich foods in the diet can alleviate micronutrient deficiency, thus preventing the appearance of chronic diseases.

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Capítulo 3

Capítulo 4

Phenolics and Antioxidant Activity of Green and Red Sweet Peppers from Organic and Conventional Agriculture: A Comparative Study

Capítulo 4

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Abstract

Today, consumers are very concerned regarding food quality, nutritional composition and positive health effects of consumed foods. In this context, the preference and consumption of organic products has been increasing worldwide. In the present work, sweet peppers in two maturation stages (i.e., green and red peppers) from organic and conventional production systems were evaluated in regards to phenolic composition and antioxidant activity. Nine phenolic compounds were identified and quantified by a high-performance liquid chromatography-diode-array detector (HPLC-DAD), namely resveratrol, meta-coumaric acid, ortho-coumaric acid, clorogenic acid, caffeic acid, myricetin, rutin, luteolin-7-O-glucoside and quercitin-3-O-rhamnoside. In contrast to the production system, the maturation stage showed a pronounced significant effect on the phenolic composition of the studied sweet peppers; in general, green peppers possessed higher contents than red ones. Meta-coumaric acid, ortho-coumaric acid and quercitin-3-O-rhamnoside were more abundant in green conventional peppers and chlorogenic acid, caffeic acid and rutin were found in higher levels in red organic peppers. Regarding the antioxidant activity, green conventional peppers showed the highest DPPH, ABTS•+ and total reducing capacities, while red conventional peppers had higher TEAC values. Finally, principal component analysis showed that the phenolic composition together with the antioxidant capacities could be used to differentiate the production system and the maturation stage of sweet peppers. This finding confirmed that both factors influenced the peppers' phenolic composition and antioxidant capacity, allowing their possible use as maturation–production biomarkers.

Keywords: phenolic compounds; resveratrol; linear discriminant analysis; production-maturation mode discrimination.

Capítulo 4

4.1. Introduction

Sweet pepper (*Capsicum annuum* L.), is one of the most popular and consumed vegetable around the world. The high diversity of fruit forms and colors, in many cases related with its maturation degree, and also its pungency, specific taste and/or distinct aroma makes sweet peppers very popular and an excellent ingredient to be included in many types of diets and dishes with high attractiveness for several type of consumers (Eggink et al., 2012).

In the last decades, there is an increasing concern by consumers for healthier, safer and high quality foods produced under environmental friendly practices and economically fair modes. In this sense, the worldwide demand for organic products has being increasingly, being expected a sharper increase in the coming years (Massey et al., 2018; Willer & Lernoud, 2019; Denver et al., 2019). Consumers believe that organics products are of better quality, tastiest, with high amount of vitamins and other healthy compounds and consequently more nutritious, and these perceptions are the main driver of the observed increase preference by organic products (Jensen et al., 2013). This perception is usually related to the fact that the use of chemical fertilizers or synthetic plant protect products are not permitted in organic farming (CR, 2007; Baudry et al., 2017; Hallmann et al., 2019), being in-line with the reported higher levels of bioactive compounds reported for organic compared to conventional sweet peppers (Hallmann et al, 2019; Barański et al., 2014). Sweet peppers are, in general, recognized as a potential food source of vitamins, phenolic compounds, carotenoids, and flavonoids, which possess known positive health effects (Hallmann et al., 2019; Cisternas-Jamet et al., 2019). Besides the agronomic production mode (Ritota et al., 2010; Sim & Sil, 2008; Sun et al., 2007; Del Amor et al., 2008; Flores et al., 2013; Guclu et al., 2021), the richness in bioactive compounds (e.g., carotenoids and phenolic compounds) and the related antioxidant capacities, greatly depends on the sweet peppers' cultivar (Chassy et al., 2006; Morales-Soto et al., 2013; Materska, 2014; Lekala et al., 2019) and on the fruits' maturation stage (Cisternas-Jamet et al., 2019; Alu´datt et al., 2019). Several phenolic compounds have been detected and quantified in sweet peppers. The phenolics have been detected in both free and bound forms (Lekala et al., 2019). Depending on the cultivar, production mode, maturation stage, environmental-climatic conditions and geographical origin, several phenolics have been found, including flavonoids and hydroxycinnamic acids. In fact, apigenin, caffeic, chlorogenic, ferulic, p-courmaric, p-hydroxybenzoic, rosmarinic, sinapic and vanillic, acids, naringenin, quercetin-3-O-glucoside, and luteolin have been found in different levels, being not always consensual the effect of some of the above mentioned factors (Guclu et al., 2021; Morales-Soto et al., 2013; Materska, 2014; Lekala et al., 2019; Alu´datt et al., 2019; Marín et al., 2008; Hallmann & Rembialkowska, 2012; Zhuang et al., 2012; Rodrigues et al., 2019; Fratianni et al., 2020; Kelebek et al., 2020).

Therefore, it still is of utmost interest to assess the specific phenolic composition of different sweet pepper cultivars, as well as to establish the possible effects of the production mode and maturation stage. Indeed, it has been previously reported that the phenolic profiles alone or coupled with other chemical data (e.g., volatile fraction composition, antioxidant activity data, among others) allowed differentiating, peppers' cultivars (Fratianni et al., 2020; Sora et al., 2015), red pepper cultivars grown under different shade and controlled-temperature conditions (Lekala et al., 2019), fresh and cooked sweet peppers of two cultivars (Kelebek et al., 2020), sweet and hot peppers (Valle et al., 2020) as well as fresh and dried red peppers grown under conventional or organic systems (Guclu et al., 2021). Recently, the research team has shown that chemical-sensory data and potentiometric signal profiles, recorded using a lab-made electronic tongue could be satisfactorily used as biomarkers of sweet peppers' agronomic production system (organic versus conventional) and the maturation stage (green versus red fruits) (Guilherme et al., 2020). In the present work, we intended to evaluate the effect of the production system (organic and conventional) and maturation stage (green and red colors) on the phenolic composition and on the antioxidant activity of sweet peppers from the Entinas variety. These effects were further evaluated based on unsupervised (principal component analysis, PCA) and/or supervised (linear discriminant analysis, LDA) pattern recognition techniques. To limit/overcome the known influence of non-controlled external factors (e.g., sweet pepper varieties, agro-climatic conditions, soil compositions, harvest time-periods, among others), the study was limited to a single pepper variety grown by two producers within the same geographical area. This option allowed a deep evaluation of the two factors under study (production system and maturation stage), although it posed some additional difficulties in establishing an optimal and general study model.

4.2. Materials and Methods

4.2.1. Sweet peppers production system and sampling

For this study two different sweet peppers (*Capsicum annuum* L.) producers were selected. Both producers, one organic and one conventional, are located near Coimbra, in the center region of Portugal. Organic producer followed the organic production European Commission guidelines (CR, 2007), and conventional producer, followed the conventional roles of agricultural production without the limitations of use of pesticides and fertilizers. The peppers' producers were selected taking into account some aspects, namely, soil with similar physical and chemical characteristics. Soil of both fields was analyzed before the experiment. The organic field presented a pH value of 6.4, organic matter 1.5%, 105 mg P_2O_5 kg^{-1} (available P) and 156 mg K_2O kg^{-1} (available K), whereas the values for the conventional field were 6.0 for pH, 1.8% for organic matter, 181 mg P_2O_5 kg^{-1} for available P and 134 mg K_2O kg^{-1} for available K. The production occurred in open field conditions. The planting of sweet pepper seedlings, from the Entinas variety, was performed in the last days of May 2018.

The number of plants per hectare was 22 222 (75 cm between rows, 60 between plants). In both fields, plants were drip-irrigated with water from Mondego River, with a flow rate of 2.66 L s^{-1} per emitter during 20 min every two or three days according to the climatic conditions. In the organic field, nutritional requirements were supplied by the incorporation of 29.6 t ha^{-1} (5.8 g of N kg^{-1}, 2.8 g P_2O_5 kg−1 and 5.3 g K_2O kg^{-1}) of horse manure on soil before planting. In the conventional field, chemical fertilizers were used at a rate of 700 kg ha^{-1} before sweet pepper planting. The fertilizer was composed of 7% N, 14% P_2O_5, 14% K_2O, 3% CaO, 2% MgO, 9% SO_3 and 0.02% B; this was reinforced in late July with 300 kg ha^{-1} of a fertilizer composed of 27% N and 4% CaO. A phytosanitary treatment was applied against aphids (100 g L^{-1} of deltamethrin) in the conventional field.

The harvest of sweet peppers occurred in the second week of September 2018, and from each production system (organic and conventional) two different maturation stages were selected. The first, green, corresponds to completely developed fruits that have reached the requirements to be collected; and the red corresponds to an advancement of maturation when fruits are completely red. For each production system and each color, five independent 2 kg samples were collected, for a total of 20 independent samples. Afterward, the fruits were transported to the laboratory, washed, had all non-edible parts removed, frozen at −20 °C and lyophilized until analysis.

4.2.2. Sweet Peppers Phenolic Compounds Evaluation by High-Performance Liquid Chromatography-Diode-Array Detector (HPLC-DAD)

The evaluation of the phenolic composition of sweet pepper samples was performed using a solid–liquid extraction followed by a high-performance liquid chromatography (HPLC) -diode-array detector (DAD) injection to identify each phenolic present in each sample. The extraction of phenolic samples was performed by mixing 40 mg dried powder (dw) of each sample with 950 μL of 70% methanol and 50 μL of internal standard (naringin). Each mixture was agitated thoroughly in a vortex and placed in a thermoblock at 70 °C for 45 min, and then centrifuged (Centrifuge 5804 R, Eppendorf, Hamburg, Germany) at 4000 rpm for 15 min. The extracts were then filtered (Fisherbrand Ø 90 mm) and supernatants transferred to amber vials and stored at −20 °C (Chromacol 2-SVWK(A)ST-CPK, ThermoScientific, Langerwehe, Germany) until further analysis.

The HPLC-DAD system used was a Gilson HPLC (Villers-le-bel, France) equipped with a Finnigan/Surveyor DAD (Thermo Electron, San Jose, CA, USA, C18 column (250 × 4.6 mm, 5 μm) (ACE, Aberdeen, Scotland) with an eluent composed of water with 0.1% of trifluoroacetic acid (TFA) (solvent A) and acetonitrile with 0.1% TFA (solvent B) and a flow rate of 1 mL min^{-1}. The gradient used started from 0% solvent B at 0 min, 0% solvent B at 5 min, 20% solvent B at 15 min, 50% solvent B at 30 min, 100% solvent B at 45 min, 100% solvent B at 50 min, 0% solvent B at 55 min and 0% solvent B at 60 min.

The chromatograms were recorded at 254, 280, 320 and 370 nm. The identification of phenolics was based on their peak retention times, UV spectra and UV max absorbance bands in comparison with commercial standards (resveratrol, meta-coumaric acid, ortho-coumaric acid, chlorogenic acid, caffeic acid, myricetin, rutin, luteolin-7-O-glucoside, quercitin-3-O-ramnoside; Extrasynthese, Genay, Rhône, France) and literature. Phenolics were quantified using the internal standard method and the results expressed in μg g^{-1} dw as the mean ± standard error of three replicates.

4.2.3. Sweet Peppers Antioxidant Activity Assays

4.2.3.1. Preparation of extracts

The different samples were split according to the production system (organic and conventional) and maturation stage (green and red). All peppers were washed, cut, cleaned of seeds, frozen in plastic bags and then freeze-dried. Afterward, the extraction was performed using 600 mg of freeze-dried sample in 150 mL of water–methanol solution (70:30, v/v), at 70 °C for 45 min. Afterward, methanol was removed using a rotary evaporator (Stuart Re300), and the remaining water was removed by freeze-drying until a dry extract was obtained. Typical electron transfer antioxidant-based assays were performed, including DPPH, ABTS•+ and TEAC.

4.2.3.2. Determination of the Blocking Effect of 2,2-Diphenyl-1-Picrilhhydrazyl Free Radicals (DPPH)

The evaluation method used to detect the ability to block DPPH free radicals from pepper extracts was described by Hatano et al. (1988). Thus, 0.3 mL of extract with predetermined concentrations of each sample was mixed with 2.7 mL of a methanolic solution containing DPPH radicals (6 x 10-5 mol/L) (Sigma-Aldrich). The mixture was vigorously stirred and left to stand in the dark at room temperature for 60 minutes, until stable absorbance values were obtained. The spectrophotometric reading was done at 517 nm in a UV-Visible UV-1280 spectrophotometer model Shimadzu and the results were presented in percentage of DPPH discoloration, using the following equation:

% Blocking effect = [(ADPPH-AA)/ADPPH] x 100

The antioxidant activity values corresponding to the absorbance of the solution with the sample extract and the ADPPH values the absorbance of the DPPH solution (white).

4.2.3.3. Radical scavenging activity (ABTS•+)

The formation of the ABTS radical [2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)] is the basis of one of the spectrophotometric methods that has been applied to measure the antioxidant activity of products. The pepper radical scavenging activity was carried out following the method of Sánchez et al. (2007) with some modifications described below. To prepare the solution, 25 mL of ABTS•+ solution. The radical was generated using 0.440 mL of the potassium persulfate solution, where after stirring it was placed in the dark for 12 to 16 hours. The solution was diluted with absolute ethanol until an absorbance of 0.70 ± 0.02 was obtained, read at λ = 734nm. Once the radical was formed, 2 ml of ABTS•+ solution was mixed with 0.1 ml of the sample with the previously determined concentration. After 6 minutes of reaction, each sample was read at 734nm on a Shimadzu UV-Visible UV-1280 spectrophotometer. The results were presented in percentage of ABTS•+ discoloration using the same equation presented in the free radical blocking effect (DPPH) method.

4.2.3.4. Reducing Power

To perform the assessment of the reducing power of the extracts, the method described by Berker et al. (2007). Thus, 1 mL of the extract solution was used, 2.5 mL of 0.2 M sodium phosphate buffer solution with a pH of 6.6 and 2.5 mL of potassium ferrocyanide ($K3Fe(CN)6$) to 1%. The formed mixture was stirred and incubated at 50 °C for 20 minutes in a water bath. After cooling the samples, 2.5 ml of 10% (w/v) trichloroacetic acid was added, stirring vigorously. 2.5 ml of the mixture supernatant was removed and 2.5 ml of distilled water and 0.5 ml of 0.1% iron (III) chloride were added. After the mixture with all the necessary reagents was ready, it was waited 2 minutes and the reading to assess the reducing capacity was made at 700 nm absorbance. The results obtained were expressed in mg Trolox per g of sample.

4.2.4. Statistical analysis

One-way ANOVA was applied to discuss the statistical significance of the agronomic production system–maturation stage effect on the individual and total phenolic composition as well as on the antioxidant radical scavenging activity. In the case that a statistically significant effect was detected (i.e., p-value < 0.05) the post-hoc multi-comparison Tukey’s test was further used to identify which levels were or were not significantly different. Boxplots were used to visualize the statistical results. As usual, the 1st, 2nd (median) and 3rd quartiles were plotted and the box bars represented the values that are within the 1st and 3rd quartiles. Additionally, whiskers were plotted (vertical lines) from the middle of the top and bottom edges of each box. The whiskers were 1.5 times the inner quartile spread in length, being measured from the median. The whiskers provided an arbitrary cutoff point to identify data points that were possible outside values. Minimum and maximum values that fell outside the whisker range were plotted (dot symbols) and symbolized possible extreme values or outliers.

In addition, the differentiation/discrimination of the agronomic production system and/or fruit’s maturation stage was also evaluated (unsupervised and supervised classifications) using, respectively, the principal component analysis (PCA) or the linear discriminant analysis (LDA). The unsupervised differentiation performance was evaluated using 3D-plots of the first three principal components (PCs). The supervised classification technique was implemented together with the simulated annealing (SA) algorithm (i.e., a variable selection meta-heuristic algorithm) to choose the non-redundant variables with the most discrimination potential and to minimize noise effects (Bertsimas & Tsitsiklis, 1993; Cadima et al., 2004). The predictive performances of the LDA-SA models were checked using two cross-validation (CV) variants, namely the leave-one-out cross-validation (LOO-CV) and the repeated K-fold-CV (with 10 repeats and K set equal to 4, allowing that 25% of the data were used for validation purposes at each iteration). For both variants, the percentage of correct classifications (i.e., the model's sensitivity) was calculated. The statistical analysis was performed using the Subselect (Cadima et al., 2004) and MASS (Venables & Ripley, 2002) packages of the open source statistical program R (version 2.15.1), at a 5% significance level.

4.3. Results and Discussion

4.3.1. Phenolic composition of Entinas sweet peppers

Nine phenolic compounds (caffeic, chlorogenic, m-coumaric and o-coumaric acids, luteolin-7-O-glucoside, myricetin, resveratrol, rutin and quercetin-3-O-rhamnoside) were detected, by HPLC-DAD, in all the studied Entinas sweet pepper samples grown under both conventional and organic modes and at the two maturation stages (green and red peppers). The contents (in μg g^{-1} dw) of the nine phenolic compounds quantified in Entinas peppers are shown in Figure 4.1, according to the maturation stage (green and red peppers) and the agronomic production system (conventional and organic systems). The less abundant phenolic was m-coumaric acid (red organic peppers with a mean content of 1.15 μg g^{-1} dw) and the most abundant one was luteolin-7-O-glucoside (green conventional peppers with a mean content of 458.54 μg g^{-1} dw). Although the cultivar, production system, fruits' maturation stage, environmental-climatic conditions and post-harvest treatments significantly influence the peppers' phenolic composition and contents, in general, the identified phenolics and respective levels found for the Entinas peppers were in-line with the wide range of values previously reported for other sweet pepper cultivars (Guclu et al., 2021; Materska, 2014; Lekala et al.,2019; Alu´datt et al., 2019; Hallmann & Rembialkowska, 2012; Rodrigues et al., 2019; Fratianni et al., 2020;Kelebek et al., 2020; Jeong et al., 2011). As can be visualized, for each phenolic compound, the contents varied within each production system–maturation stage group. Still, significant statistical differences (p-values < 0.05, for one-way ANOVA) were found among the contents of the individual phenolic compounds of green and red peppers grown under conventional or organic systems. Green peppers had higher contents of chlorogenic, m-coumaric and o-coumaric acids, as well as of resveratrol, myricetin, luteolin-7-O-glucoside and quercetin-3-O-rhamnoside compared to red peppers; these two latter phenolics were the most abundant ones. This finding pointed out that, with the exception of caffeic acid and rutin, maturation promoted a significant decrease of the abundance of the majority of the individual phenolic compounds. Similar trends were reported for total phenolic contents decreasing with the ripening type in different pepper cultivars (Ghasemnezhad et al., 2011; Ekinci et al., 2014). The observed decreasing trend could be tentatively attributed to the synthesis of amino acids (e.g., phenylalanine, tyrosine, among others), in the early stage of fruit ripening, which enter the metabolic pathway of the shikimic acid, acting as precursors of phenolic acid formation, leading to possible higher contents in green fruits. However, other works also showed that the phenolic content trend (decrease or increase) with the maturation stage could be cultivar-dependent (Howard et al., 2000). Indeed, an increasing trend of the total phenolic contents (TPC) with the ripening time was described for green and red bell peppers (Cisternas-Jamet et al., 2020).

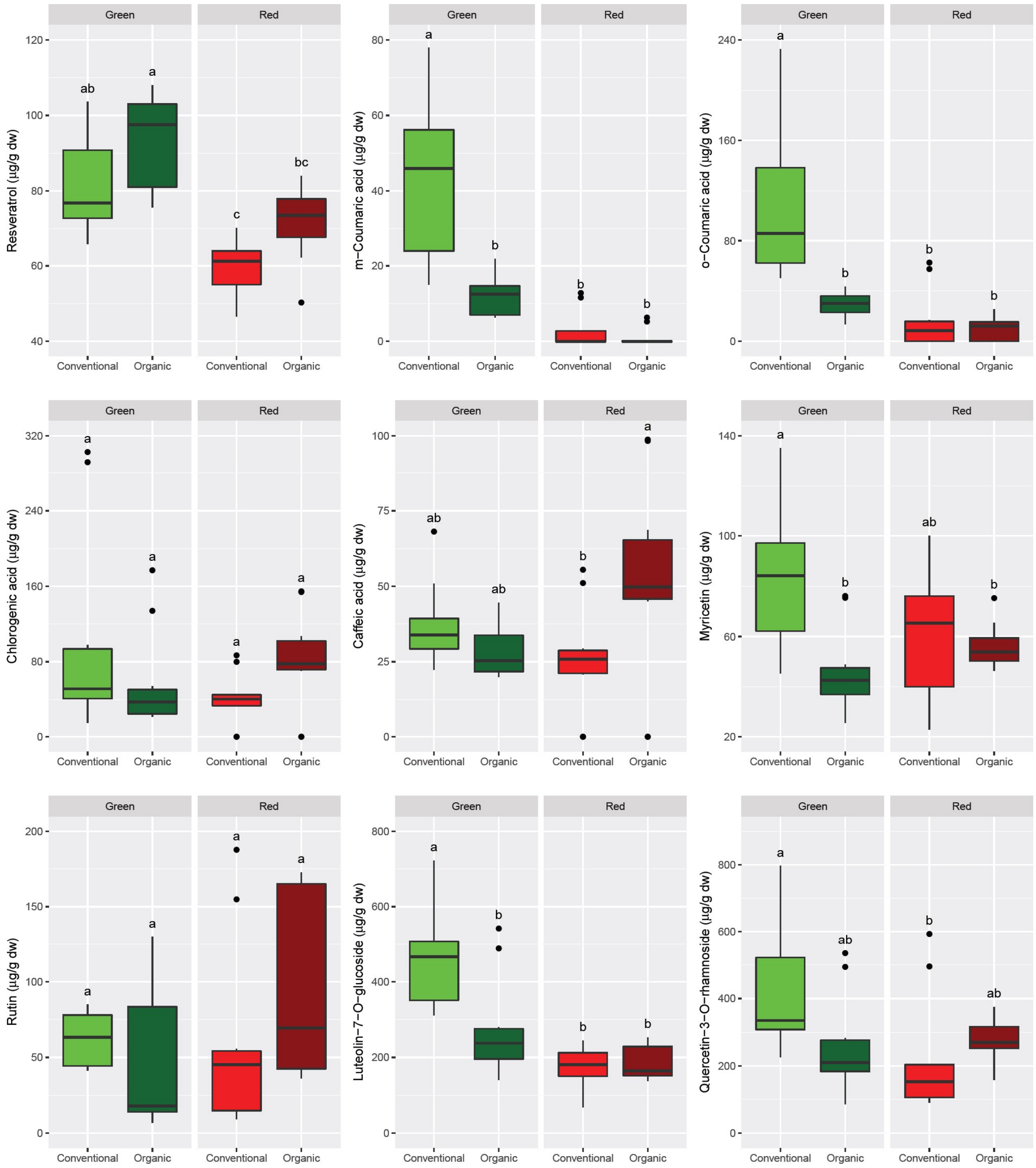

Figure 4.1. Individual phenolic compounds quantified (in µg g^{-1} dw), by High-Performance Liquid Chromatography-Diode-Array Detector (HPLC-DAD), in sweet pepper samples according to the maturation stage (green or red peppers) and agronomic production system (conventional or organic systems). Box bars represent the values that are within the 1st and 3rd quartiles and the horizontal line represents the 2nd quartile (median). Vertical lines from the middle of the top and bottom edges of each box represent the whiskers. Dot points represent minimum or maximum values that fall outside the whisker range (extreme values or outliers). Different lower case letters mean significant statistical differences (p-value < 0.05) among maturation–production levels.

On the other hand, although several studies reported that peppers grown under the organic system were richer in phenolics, namely in total phenol content compared to those grown under the conventional system (Hallmann et al., 2019; Barański et al., 2014; Del Amor et al., 2008; Guclu et al, 2021), he results of the present study (Figure 1) show that, sometimes, conventional peppers possessed greater contents of some individual phenolic compound.

In fact, Entinas peppers produced in the conventional system had higher contents of m-coumaric and o-coumaric acids, myricetin, luteolin-7-O-glucoside and quercetin-3-O-rhamnoside, but lower contents of caffeic acid, resveratrol and rutin, compared to those grown under the organic system. However, Marín et al. (2008) observed slight differences among the contents of individual and total phenolic compounds of green and red peppers cultivated under organic, integrated or soil-less systems.

The opposite findings reported in the literature, together with those of the present study clearly show the difficulty in attempting to establish a priori the effects of the agronomic production system and/or maturation stage on the phenolic composition of sweet peppers, pointing out the relevance of factors such as cultivar, environmental-climatic conditions and post-harvest treatments. If an optimal and general evaluation model was envisaged, a broader study would be needed, which should take into account different sweet pepper varieties grown in different geographical regions under different agronomic practices and subjected to different climatic conditions. However, several evaluation difficulties would arise from such a wide-ranging approach, leading to a complex data analysis where main conclusions could be hard to identify and further extrapolate to other practical cases.

4.3.2. Antioxidant Activity and Total Phenolic Content of Entinas Sweet Peppers

The total phenolic contents (TPC), calculated as the sum of the individual contents of the detected phenolics, as well as the antioxidant activities (DPPH, in %; ABTS•+, in %; and TEAC, in mg Trolox/g) of the Entinas sweet peppers, are shown in Figure 4.2, according to the production mode (conventional and organic) and maturation stage (green and red). The mean TPC values varied from 656 to 1 400 µg/g dw for red and green conventional peppers, respectively, which are in agreement with the wide range of literature values determined using either spectrophotometric (Folin–Ciocalteu based-method: 300-7 700 µg/g) (Alu´datt et al., 2019; Kelebek et al., 2020; Cisternas-Jamet et al., 2020; Lutz et al., 2015; Hamed et al., 2019) or chromatographic assays (LC coupled or not with MS: 120-4 000 µg/g) (Guclu et al., 2021; Lekala et al., 2019; Kelebek et al., 2020). These values greatly depend on several factors, including, pepper cultivar, production system, environmental-climatic conditions, maturation stage and post-harvest treatments. Similarly, red and green conventional peppers showed, respectively, the lowest and highest mean DPPH (varying from 55% to 71%) and ABTS•+ (ranging between 45% and 61%) radical scavenging activities. An opposite finding was observed for TEAC, for which red conventional peppers had the greater values (11 mg Trolox g^{-1}) and green conventional peppers had the lower ones (8 mg Trolox g^{-1}). It should be noticed that the DPPH values found are in accordance with the values reported by several research teams (varying from 45% to 87%) for different pepper cultivars at different maturation stages and grown under different agronomic sytems (Hamed et al., 2019; Zhang et al., 2003; Wong & Tan, 2020).

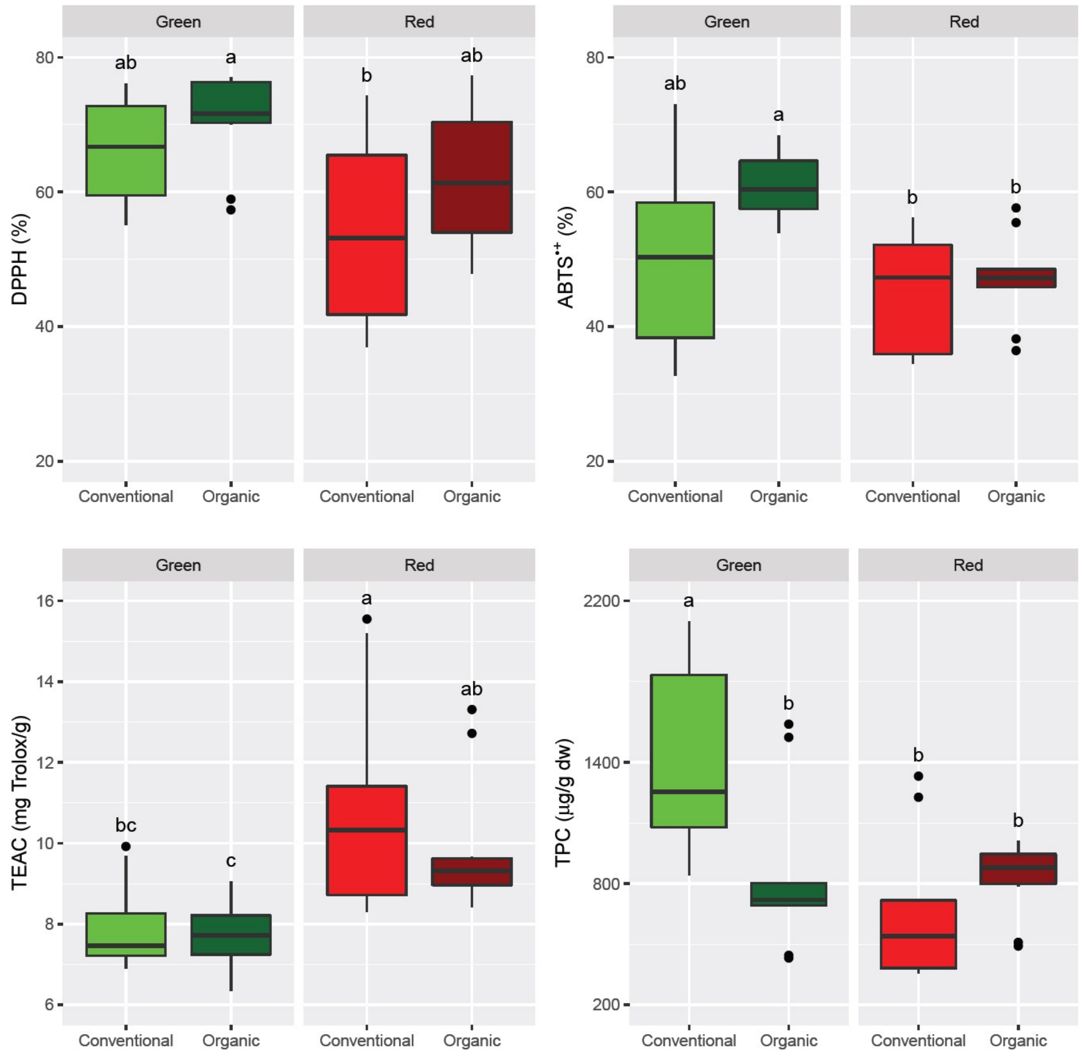

Figure 4.2. Antioxidant activities (DPPH, in %; ABTS•+, in %; and, TEAC, in mg Trolox/g) and TPC (sum of individual phenolics, in µg/g dw) of sweet peppers according to the maturation stage (green or red peppers) and agronomic production mode (conventional or organic modes). Different lower case letters mean significant statistical differences (P-value < 0.05) among maturation-production levels.

Overall, green peppers had significant greater DPPH and ABTS•+ activities compared to red peppers, probably due to the higher TPC, but showed lower TEAC. Thus, the TPC and antioxidant activity were favored by early maturation stage of Entinas peppers. Oppositely, Lutz et al. (2015) and Cisternas-Jamet et al. (2020) observed an increase in the DPPH activity with ripening. Regarding the oxygen radical antioxidant capacity (ORAC), a similar increasing trend was found by Lutz et al. (2015), although no clear effect of maturity was observed by Cisternas-Jamet et al. (2020). In addition, Martí et al. (2011) did not found significant changes in the total antioxidant activity between green and red peppers of different cultivars. It should be noted that DPPH and ABTS•+ radical scavenging activity as well as the ferric reducing antioxidant power assay (FRAP) are greatly cultivar-dependent (Alu´datt et al., 2019; Kelebek et al 2020; Hamed et al., 2019; Aslani et al., 2016; Alam et al., 2020), which can partially explain the different trends found in the present study as well as in the literature. Concerning the agronomic production system effect on the radical scavenging activity, conventional peppers showed the highest TPC and TEAC but lower DPPH and ABTS•+ activities, compared to organic peppers.

4.3.3. Unsupervised and supervised Entinas peppers differentiation based on the phenolic profile and antioxidant radical scavening data

The possible use of the phenolic composition (individual and total contents) and/or the related radical scavenging activity (DPPH, ABTS•+ and TEAC) data to act as possible biomarkers of Entinas peppers production system/maturation stage was further evaluated using unsupervised (PCA) and supervised (LDA) multivariate pattern recognition techniques. Previously these statistical techniques have been applied, for example, to differentiate pepper cultivars/maturation stage based on the phenolic composition (Fratianni et al., 2020), although different cultivars/maturation stages were grouped into the same clusters; or to distinguish fresh and dried peppers grown under conventional or organic systems (Guclu et al., 2021), although in this case, the use of both phenolic and aroma compounds was required. Recently, the production system–maturation stage of Entinas peppers could be identified using chemical-sensory data or potentiometric signal profiles, recorded using a lab-made taste sensor device (Guilherme et al., 2020). As reported by Guilherme et al. (2020), LDA classification models could be established using non-redundant independent parameters selected by the simulated annealing (SA) algorithm, allowing the correct classification of 80–90% of the studied samples (leave-one-out cross-validation, LOO-CV). In the present study, the possibility of assessing the production system–maturation stage of Entinas peppers, based on the phenolic composition and antioxidant capacity was further evaluated using PCA and LDA-SA approaches.

Figure 4.3 shows the 3D-PCA plots based on the individual (caffeic, chlorogenic, m-coumaric and O-coumaric acids, luteolin-7-O-glucoside, myricetin, resveratrol, rutin and quercetin-3-O-rhamnoside) and total phenolic contents (TPC) together with the radical scavenging activities (DPPH, ABTS•+ and TEAC). As can be inferred, the three first principal components (1st, 2nd and 3rd PCs), which explained 80% of the total data variance, allowed a satisfactory differentiation of the studied Entinas peppers either considering simultaneously the production system–maturation stage (Figure 4.3a), or taking into account each factor separately (Figure 4.3b,c, for production system or maturation stage, respectively). Moreover, from a qualitative point of view, it is also clear that the abovementioned data would enable a better recognition of the maturation stage compared to the production system, pointing out that the phenolics composition and related antioxidant activities are more influenced by peppers' maturation. To identify the parameters that had the higher discriminant power, LDA-SA models were established for each aim, i.e., to simultaneously discriminate the production system–maturation stage or each factor per se.

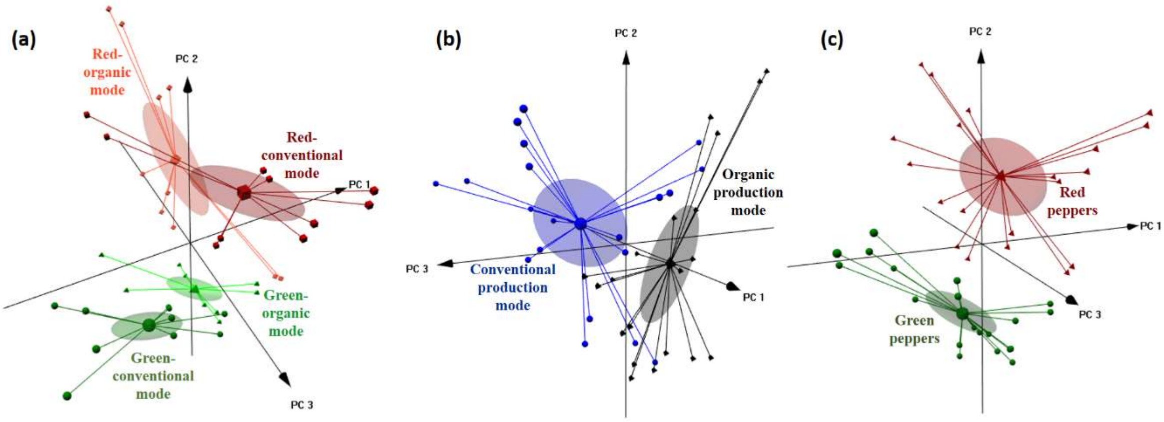

Figure 4.3. Unsupervised differentiation (3D-PCA plots) of Entinas sweet peppers based on the nine individual phenolics detected by HPLC-DAD (caffeic, chlorogenic, m-coumaric and o-coumaric acids, luteolin-7-O-glucoside, myricetin, resveratrol, rutin and quercetin-3-O-rhamnoside, µg/g dw), total phenolic content (TPC, µg/g dw) and radical scavenging activity data (DPPH, in %; ABTS•+, in %; and, TEAC, in mg Trolox/g): (a) according to the agronomic production mode (organic and conventional) and the maturation stage (green and red peppers); (b) according to the agronomic production mode (organic and conventional) independently of the maturation stage; (c) according to the maturation stage (green and red peppers) independently of the agronomic production mode.

For the simultaneous discrimination of the four production system–maturation stage levels a LDA-SA model with three discriminant functions was established based on seven non-redundant parameters (resveratrol, m-coumaric acid, chlorogenic acid, myricetin, quercetin-3-O-rhamnoside, TPC and TEAC) selected by the SA algorithm. The classification model allowed the correct classification of 100%, 75% and 76 ± 16% of the peppers, for the original data grouped (training), LOO-CV and repeated K-fold-CV (predictive internal validation variants). Although 5 of 20 peppers were misclassified, it should be remarked that, the misclassification occurred between the production system within the same maturation stage (i.e., green and red peppers were always correctly classified).

Therefore, to further confirm this finding, LDA-SA models were also developed for predicting the peppers' production system or maturation stage. For the production system, classification models with one discriminant function were obtained based on four non-redundant parameters (o-coumaric acid, chlorogenic acid, myricetin and DPPH). This model had sensitivities (i.e., correct classifications) of 100%, 90% and 89 ± 11% for the original data grouped, LOO-CV and repeated K-fold-CV. In this case, only 2 of the 20 peppers were misclassified, with one sample of each production system misclassified. Finally, for the maturation stage, a model with one discriminant function was also established based on only three phenolic compounds (resveratrol, m-coumaric acid and myricetin), which allowed 100% of correct classifications for training and both predictive cross-validation variants. The overall results clearly show that maturation stage could be easily predicted based on the peppers' phenolic composition; this was also reliable for the assessment of the production system. Thus, phenolic and antioxidant data could be used as a preliminary peppers classification tool. However, for taking into account both factors simultaneously (i.e., production system and maturation stage) a data fusion approach, using other physicochemical data would be required.

4.5. Conclusions

The study carried out confirmed that the production system, as well as the maturation stage has a significant effect on the phenolic profile and on the antioxidant capacity of Entinas sweet peppers. Regarding the Entinas cultivar, it was observed that a lower maturation degree (i.e., green peppers) promoted the increase of the total phenolic content, the DPPH-radical scavenging activity and the bleaching of the ABTS radical cation, but a lower TEAC. Regarding the individual phenolic levels, the maturation effect greatly depended on the specific phenolic compound. Concerning the agronomic production system, the conventional system seemed to enhance the overall phenolic-antioxidant richness of the studied Entinas peppers, although this trend was not observed for all individual phenolics. Finally, the chemometric evaluation performed allowed us to verify that Entinas peppers' phenolic-antioxidant levels could be satisfactorily used as discrimination biomarkers for both production system and maturation stage; this finding is more visible for the maturation stage recognition. Nevertheless, it should be emphasized that the abovementioned conclusions were established for a specific sweet pepper variety grown within a narrow geographical region and so, any extrapolation should be carefully made if different varieties grown under different agro-climatic conditions are considered in the future. Indeed, to establish an optimal and general model, other external factors must be included in a future study.

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Capítulo 5

Sweet pepper, *Capsicum annuun* L., volatiles: effect of producing mode and maturation stage

Capítulo 5

Referência - Capítulo em preparação para submissão:

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Capítulo 5

Abstract

Nowadays, consumers are increasingly aware about the benefits of healthy diets that include vegetables and fruits with a recognized positive effect on health and that are produced according to organic production modes, following ecofriendly practices. Sweet pepper (*Capsycum annuun* L.) is a very appreciated vegetable consumed as raw, grilled, cooked or in other different preparations. Peppers' volatile composition may influence consumer's preference and could be related with fruit maturation stage and production mode. In this sense, in this work the effect of the maturation stage (green or red) and production mode (organic and conventional) on volatile composition of raw fresh sweet peppers (Entinas variety) was studied. The chemical characterization of the volatile fraction of sweet pepper allowed the identification of fourty-eight compounds belong to the following chemical groups: alcohols, aldehydes, alkanes, esters, hydrocarbons, ketones, sesquiterpenes and terpenes. Twenty of the identified volatiles were found in all analysed peppers, namelly 2-heptanone; 2-methoxy-3-(2-methylpropyl-)-pyrazine; 6-methyl-5-hepten-2-one; cis-β-Ocimene; copaene; decanal; dodecane; hexadecane; limonene; linalool; methyl octanoate; myrcene; nonanal; nonanol; p-cymene; tetradecane; trans-β-Ocimene; α-pinene; β-Elemene e γ-terpinene. In green peppers, a higher number of volatiles could be detected compared to the red ones and, for both maturation stages, the peppers produced according to conventional agriculture also presented a higher number of compounds when compared to pepper from organic agriculture. Indeed, the peppers' different volatile profiles allowed the unsupervised (principal component analysis) and the supervised (linear discriminant analysis) differentiation of the studied samples according to the production mode. The supervised analysis also pointed out that, the volatile compounds with most discriminant power varied according to the pepper maturation stage being myrecene, neo-allo-ocimene, γ-terpinene and 2-methoxy-3,2-methypropyl-pyazine in green peppers and nonanol, p-cymene and dodecane in red ones, those that mostly contributed for the production mode pattern recognition.

Keywords: green and red sweet peppers, volatile composition, organic agriculture, linear discriminant analysis.

Capítulo 5

5. 1 – Introduction

The sweet peppers (*Capsicum annuum* L.) fruits are worldwide consumed in fresh or dried forms, being highly appreciated by consumers due to their diversified forms, colours, flavours and nutritional quality. They are produced worldwide and are used as food ingredients (Eggink et al., 2012). Also, they are often used as flavouring, colorant and add tang and taste to otherwise insipid foods (Pugliese et al., 2013).

They are cultivated under conventional or organic farming modes, being the latter based on sustainable and environmentally practices, where the use of pesticides and synthetic fertilizers is forbidden. An increasing worldwide demand for organic products has been observed, mainly due to the consumers growing concerns with health and environmental issues (Massey et al., 2018; Willer & Lernoud, 2018; Denver et al., 2019).

Sweet peppers are rich in capsaicinoids, carotenoids, flavonoids and volatile compounds, which is related to the capability of the fruit to eliminate insipidity, produce aromas and act against oxidative diseases, promoting the use within the food and pharmacological industry (Antonio et al., 2018). Peppers also contain water, cellulose, vitamins (A, B, B2, provitamin A and secondary metabolites). It was found that raw pepper contains five to seven times more vitamin C than a lemon (Ciulu-Costinescu et al., 2015). However, the composition and concentrations can vary according to the amount of sunlight, soil, season, crop region, temperature changes, variety of fruit and maturity level.

Fresh and processed peppers of different varieties have different colours, varying from green and white (unripe fruits) to yellow, orange and red (ripe fruits), corresponding to distinct stages of maturation; different sweet and spicy levels; as well as, different shapes, from large to thin fruits (Baenas et al., 2019). The quality of sweet peppers as well as of other fruits and vegetables can be established taking into account physical, chemical and sensory attributes such as appearance, flavour, texture and nutritional value (Rocha et al., 2013). Several research studies have focused on peppers' quality characteristics and their evolution during storage or due to different agronomic practices. The chemical composition, namely volatile and/or non-volatile compounds and the antioxidant capacity of cultivated or wild species has been highlighted and used for variety identification, geographical origin assessment and/or postharvest chemical/sensory evaluation (Eggink et al., 2012; Barzegar et al., 2018; Korkmaz et al., 2020). The flavour characteristics are highly dependent on the fruit variety but also on the growing conditions/production modes (e.g., conventional or organic practices) (Hallmann, 2012; Hallmann et al., 2019) and on the fruit's maturation stage (Cisternas-Jamet et al., 2020). The high level of production and consumption of peppers throughout the world is mainly due to its chemical beneficial composition for human health (Taiti et al., 2019).

Flavour composition has been defined as a complex attribute of quality, in which the mix of sugars, acids, and volatiles play a primary role (Defilippi et al, 2009). Volatile compounds are key elements in determining fruit quality and consumers' acceptance (Legua et al., 2017). In addition to the four basic flavours (sweet, sour, salty, and bitter) that humans can recognize in fruits and vegetables, aroma has an important influence on the final consumer acceptance of the commodity (Defilippi et al, 2009).

Consumers perceive that organic foods are of better quality, more nutritious and healthier, and these perceptions are some of the main drivers of the organic market (Jensen et al., 2013).

Given the significant increase in consumer interest in organic food products, there is a need to determine to what extent there is a scientific basis for claims made for organic produce. Studies comparing foods derived from organic and conventional growing systems were assessed for three key areas: nutritional value, sensory quality, and food safety (Bourn & Prescott, 2002).

It was found that the physicochemical composition and sensory attributes of sweet peppers are highly dependent on the agronomic production mode and on the maturation stage (Guilherme et al., 2020). The aromatic compounds of peppers are affected by several factors, related not only to the variety and the origin but also on the growth and harvest/pos-harvest conditions, such as ripening stage and fruit preservation (Defilippi et al., 2009; Junior et al., 2012; Takahashi et al., 2018).

Aroma is an important trait of fruit quality, which has gained an increasing attention in recent years (Taiti et al, 2019). There are many studies about volatile compounds and more than 125 volatile compounds in fresh and processed *Capsicum* fruits have been reported and identified, but the significance of these compounds on the aroma is not yet fully well know (Ziino et al, 2009).

Therefore, the aim of this study was to compare the volatile composition of one sweet pepper variety produced according to different agricultural practices (organic and conventional modes), and harvest at different maturation stages (green and red peppers) and thus, contributing to clarify consumers about nutritional quality of sweet peppers in those conditions.

5.2. Materials and methods

5.2.1. Sweet peppers production mode and sampling

For the present study, the sweet pepper cv. Entinas was selected from two producers (one from organic and other from conventional production systems) located in the central region of Portugal. One of the producers followed the organic production European Commission guidelines (Council Regulation (EC) nº 834/2007 of 28 June 2007) whereas the other followed conventional agriculture without any limitations on the use of pesticides. In both fields, the soil had similar acidity, with pH (H_2O) 6.0-6.4, organic matter around 1.5-2.0% and similar physical and chemical characteristics. Sweet pepper seedlings, from Entinas variety, were put in the soil in the last week of May 2018 and were grown under open field conditions. In both fields, a drip irrigation system was installed and the nutritional requirements were supplied by horse manure, in the organic field, and by chemical fertilizers, in the conventional one. In the middle of September 2018, from each producer (organic and conventional) sweet peppers were harvested at two different (increasing) maturation stages (corresponding to green, and red sweet peppers, respectively). Altogether, five independent batches, of about 2 kg each, of each agronomic production mode and maturation stage were collected, totalizing 20 independent samples. Sweet peppers were then washed, cleaned, dried, and further stored under refrigeration (~4ºC) until analysis.

5.2.2. Extraction of volatile organic compounds

Isolation of volatiles was performed by headspace solid phase microextraction (HS-SPME) and GC/MS (gas chromatography with mass spectrometry detector) according to the methodology described by Malheiro et al. (2017, 2018). Briefly, for the volatile characterization of sweet peppers were used approximately 3 g of whole fruits. All analyses were carried out in 50 mL glass vials, spiked with an accurate concentration of internal standard (4-methyl-2-pentanol) and volatiles adsorbed to an SPME fiber coated with divinylbenzene/carbonex/polydimethylsiloxane (DVB/ CAR/PDMS 50/30 μm) (Supelco, Bellefonte, USA). The sampling conditions were determined based on a conjunction of the methodologies described by Malheiro et al. (2017) and Peres et al. (2013). The samples were conditioned for 5 min at 30 °C for an incisive release of the volatile compounds. After this period, the SPME fiber was exposed during 30 min, at the same temperature, for the compounds adsorption from the headspace. Control samples (empty vials with internal standard) analyses were carried out regularly. The volatile compounds were eluted from the fiber by thermal desorption for 1 min in the injection port of the chromatograph system (220 °C). The fiber was maintained for another 10 min in the injector port for cleaning and conditioning for further analyses. The gas chromatographer used was a Shimadzu GC-2010 Plus equipped with a mass spectrometer Shimadzu GC/MS-QP2010 SE detector. A TRB-5MS (30 m × 0.25 mm × 0.25 μm) column (Teknokroma, Spain) was used. The injector was set at 220 °C and the manual injections were made in splitless mode, with helium (Praxair, Portugal) at a linear velocity of 30 cm/s and a total flow of 24.4 mL/min as mobile phase. The oven temperatures were the following: 40 °C (1 min); 2 °C/min until 220 °C (30 min). The ionization source was maintained at 250 °C with ionization energy of 70 eV, and with an ionization current of 0.1 kV. All mass spectra were acquired by electron ionization in the m/z 35–500 range. The full scan MS spectra fragments were compared with those obtained from a database (NIST 11) and with those of commercial standards acquired from diverse producers. For qualitative purposes, the areas of the chromatographic peaks were determined by integrating the reconstructed chromatogram from the full scan chromatogram using the ion base (m/z intensity 100%) for each compound. For semi-quantification purposes, volatile amounts were calculated by the ratio of each individual base ion peak area to the area of the internal standard base ion peak area and converted to mass equivalents on the basis on the internal standard mass added spectrometer Shimadzu GC/MS-QP2010 SE detector. A TRB-5MS (30 m × 0.25 mm × 0.25 μm) column (Teknokroma, Spain) was used. The injector was set at 220 °C and the manual injections were made in splitless mode, with helium (Praxair, Portugal) at a linear velocity of 30 cm/s and a total flow of 24.4 mL/min as mobile phase. temperatures were the following: 40 °C (1 min); 2 °C/min until 220 °C (30 min). The ionization source was maintained at 250 °C with ionization energy of 70 eV, and with an ionization current of 0.1 kV. All mass spectra were acquired by electron ionization in the m/z 35–500 range. The full scan MS spectra fragments were compared with those obtained from a database (NIST 11) and with those of commercial standards acquired from diverse producers. For qualitative purposes, the areas of the chromatographic peaks were determined by integrating the reconstructed chromatogram from the full scan chromatogram using the ion base (m/z intensity 100%) for each compound. For semi-quantification purposes, volatile amounts were calculated by the ratio of each individual base ion peak area to the area of the internal standard base ion peak area and converted to mass equivalents on the basis on the internal standard mass added.

5.2.3. Statistical analysis

To verify if within each maturation stage (green or red), the peppers' production mode (organic versus conventional) had or not a significant effect on the volatile profiles, the t-Student test was applied. Furthermore, principal component analysis (PCA) and linear discriminant analysis (LDA) were further used aiming to verify if the volatile profiles could be used as possible biomarkers capable to differentiate (unsupervised technique) or to discriminate (supervised techniques) the green or red peppers according to the production mode, respectively. The LDA was applied together with a meta-heuristic variable selection algorithm, i.e., the simulated annealing (SA), envisaging to identify the non-redundant most discriminant independent variables among the volatile fraction detected. All variables were scaled and centred before modelling to normalize the weight of each one on the final multivariate linear classification model. The unsupervised and supervised classification performances were qualitatively assessed using 2D plots of the principal components and, for the LDA-SA models by calculating the sensitivity values (i.e., the percentage of correctly classified samples). The statistical analysis was performed using the Subselect (Cadima et al., 2004) and MASS (Venables & Ripley, 2002) packages of the open source statistical program R (version 2.15.1), at a 5% significance level.

5.3. Results and discussion

The study carried out allowed the chromatographic identification of forty-eight volatile compounds from seven chemical families, including, alcohols, aldehydes, alkanes, esters, ketones, sesquiterpenes and terpenes, as shown in Table 6.1. It should be noticed that not all volatiles could be detected in all studied peppers, and that the found concentrations greatly depended on the maturation stage as well as on the production mode. Globally, Table 6.1 shows that for each maturation stage (green or red peppers), peppers grown under conventional mode possessed, in general, higher concentrations of volatiles compared to those grown according to the organic mode ($P < 0.05$ for the t-Student test). This finding is in-line with the data reported by Guclu et al. (2021), which also reported that the total amount of aroma compounds in red sweet peppers grown under conventional mode was higher compared to those produced under organic mode. The results from the present study also showed that the number of volatile compounds detected was higher for conventional peppers. Oppositely, Guclu et al. (2021) observed an higher number of aroma compounds in red organic peppers compared to red conventional peppers. On the other hand, for both production modes, it could also be observed that green peppers had higher amounts of the identified volatiles compared to the red peppers. Lastly, the results also pointed out that terpenes, followed by alkanes, sesquiterpenes and aldehydes were the most abundant ones for the studied Entinas peppers independently of the maturation stage or the production mode. Finally, the volatile profiles of the most abundant compounds found either in green peppers (28 volatiles) or in red peppers (22 volatiles) were further used, separately, to distinguish green or red peppers according to the production mode (conventional versus organic). The 2D-PCA biplots (Figure 6.1) clearly show that the different contents of volatile compounds chromatographically determined in green (Figure 6.1(A) and (B)) or red (Figure 6.1(C) and (D)) peppers could be used to differentiate the conventional from organic peppers, possessing the former the highest amounts (as can be inferred by the arrows direction). Furthermore, the LDA-SA approach allowed establishing supervised discrimination models, with one discriminant function that explained 100% of the data variability, that could successfully classify 100% of the samples according to the production mode for the original grouped data as well as for the leave-one-out cross-validation procedure (internal validation). The supervised approach pointed out that myrecene, neo-allo-ocimene, γ-terpinene and 2-methoxy-3,2-methypropyl-pyazine were the most powerful discriminant variables for green peppers. On the other hand, for red peppers, nonanol, p-cymene and dodecane were the most powerful discriminant variables.

Table 5.1. Volatile compounds concentration (mg kg^{-1})* of sweet pepper produced under different agricultural systems (conventional and organic) and at two maturation stages (green and red).

Nº	Compound	LRI[A]	LRI Lit[B]	QI[C]	ID[D]	Green sweet pepper Conventional	Green sweet pepper Organic	*P*-value	Red sweet pepper Conventional	Red sweet pepper Organic	*P*-value
Aldehydes											
1	Hexanal	801	801	44	S/MS	4.81±4.32	-		1.39±1.87	-	-
2	2 -heptanone	892	892	43	S/MS	5.55±6.87	6.90±6.41	0.6710	4.49±10.00	4.26±6.43	0.9514
3	6-methyl-5-hepten-2-one	988	985	43	S/MS	9.38±8.40	8.64±5.61	0.8179	3.31±2.28	4.29±1.95	0.3187
4	Nonanal	1104	1100	57	S/MS	19.04±27.97	1.87±1.59	0.0684	3.68±0.95	3.28±1.18	0.4157
5	Decanal	1206	1201	57	S/MS	1.05±1.01	0.57±0.59	0.2202	0.86±0.57	0.44±0.50	0.1027
6	Dodecanal	1410	1408	41	MS	-	-		0.26±0.55	-	
Ketones											
7	6-methyl-5-hepten-2-one	988	985	43	MS	9.38±8.40	8.64±5.61	0.8179	3.31±2.28	4.29±1.95	0.3187
Alcohols											
8	(Z)-3-hexen-1-ol	860	859	67	S/MS	-	1.25±3.84		-	-	
9	(Z)-3-Nonen-1-ol	1157	1157	41	S/MS	0.98±2.14	2.15±2.48	0.2744	-	-	
10	Nonanol	1175	1172	56	MS	4.05±5.15^{a}	0.50±0.66^{b}	***0.0444***	0.58±0.91	0.43±0.45	0.6606
11	Decanol	1274	1269	55	MS	0.28±0.68	0.15±0.32	0.5912	-	-	
Esters											
12	Methyl octanoate	1132	1127	74	S/MS	0.69±1.50	0.44±0.95	0.6708	2.12±0.34	1.86±0.41	0.1428
13	Methyl salicylate	1195	1191	120	S/MS	-	-		-	1.18±2.60	
Sesquiterpenes											
14	Copaene	1378	1376	105	MS	44.00±40.29^{a}	4.67±2.45^{b}	***0.0064***	2.51±1.56	1.29±1.58	0.0973
15	α-trans-Bergamotene	1439	1432	93	MS	36.29±48.21^{a}	0.22±0.47^{b}	***0.0294***	0.11±0.24	-	
16	Alloaromadendrene	1455	1460	91	MS	-	-		0.22±0.57	-	-
17	(E)-β-Farnesene	1461	1456	69	MS	6.71±8.72	-		-	-	
Terpenes											
18	α-pinene	938	939	93	MS	3.40±1.86	4.63±1.99	0.1678	4.84±3.84	2.57±1.37	0.0952
19	Camphene	952	954	93	S/MS	-	-		-	4.90±15.15	
20	β-pinene	979	979	93	MS	0.65±1.38	-		2.50±1.41	1.51±1.34	0.1256
21	Myrcene	993	990	41	MS	18.55±11.62^{a}	6.69±2.32^{b}	***0.0054***	6.10±1.01	9.66±15.18	0.4690

22	δ-3-carene	1007	1011	93	MS	48.47±48.53^{a}	7.82±8.19^{b}	***0.0176***	-	-	
23	p-cymene	1028	1026	119	MS	22.66±21.06	12.30±3.36	0.1420	10.62±4.06	9.10±1.29	0.2759
24	Limonene	1032	1029	68	MS	101.58±31.60^{a}	73.03±19.58^{b}	***0.0258***	89.56±21.65	73.90±10.46	0.0542
25	cis-β-Ocimene	1043	1037	93	MS	171.95±160.86^{a}	30.12±23.00^{b}	***0.0129***	2.79±0.63	2.28±0.47	0.0576
26	trans-β-Ocimene	1054	1050	93	MS	3674.78±36662.39^{a}	577.55±577.40^{b}	***0.0166***	11.29±8.07	6.75±3.99	0.1278
27	γ-terpinene	1061	1059	93	MS	80.26±75.9^{a}	11.60±15.34^{b}	***0.0118***	2.31±0.61^{a}	1.76±0.29^{b}	***0.0200***
28	Terpinolene	1084	1088	93	MS	12.90±13.6^{a}	1.02±2.29^{b}	***0.0142***	-	-	
29	p-Cymenene	1091	1091	117	MS	-	0.29±0.60		-	-	
30	Linalool	1100	1096	71	MS	43.48±47.47	15.10±9.89	0.0807	15.68±6.55	8.42±3.87	***0.0076***
31	Alloocimene	1133	1132	121	MS	219.60±285.87^{a}	33.81±33.71^{b}	***0.0132***	-	0.42±0.54	
32	trans-Limonene oxide	1141	1142	43	MS	-	-		0.11±0.23	-	
33	Neo-allo-ocimene	1145	1144	121	MS	275.18±285.87^{a}	38.10±39.09^{b}	***0.0182***	-	-	
34	Camphor	1146	1146	95	MS	-	-		4.76±1.06^{a}	3.77±0.85^{b}	***0.0331***
35	Terpinen-4-ol	1179	1177	71	MS	-	0.95±1.37		0.91±0.98	-	
36	Cyclosativene	1369	1371	105	MS	0.68±1.43	-		-	-	
37	α-ylangene	1371	1373	105	MS	0.32±0.67	-		-	-	
38	sativene	1392	1391	108	MS	0.39±0.83	-		-	-	
39	β-Elemene	1393	1389	81	MS	1.85±2.05	1.20±1.11	0.3903	1.53±2.42	1.15±1.67	0.6878
40	β-Copaene	1433	1432	161	MS	0.99±2.09	-		-	-	
41	Geranyl acetone	1456	1455	43	MS	-	-		0.58±1.32	-	
42	Germacreme D	1481	1484	161	MS	0.50±1.07					
43	Valencene	1494	1496	161	MS	-	-		0.21±0.57	-	
Alkanes					MS						
44	Dodecane	1200	1201	57	S/MS	43.65±35.28^{a}	9.36±10.94^{b}	***0.0088***	8.27±3.43^{a}	1.31±0.83^{b}	***<0.0001***
45	Tridecane	1301	1300	57	MS	0.72±1.03	-		-	-	
46	Tetradecane	1400	1400	57	MS	42.62±33.07^{a}	5.60±7.38^{b}	***0.0028***	10.63±5.94^{a}	1.79±1.97^{b}	***0.0003***
47	Pentadecane	1502	1500	57	S/MS	12.58±20.55	-		-	-	-
48	Hexadecane	1601	1600	57	S/MS	18.03±23.63^{a}	1.23±1.63^{b}	***0.0378***	2.12±1.53	0.18±0.38	***0.0010***

*Values are from semi-quantification using 4-methyl-2-pentanol as internal standard; [A]LRI – Linear retention index obtained; [B]LRI Lit – Linear retention index reported in literature (Adams, 2007); [C]Quantification ion; [D]Identification method (S – identified with standard; MS – identified by comparing mass spectrum with database NIST 11); tr. – below 0.001 mg kg^{-1}; $^{a-}$

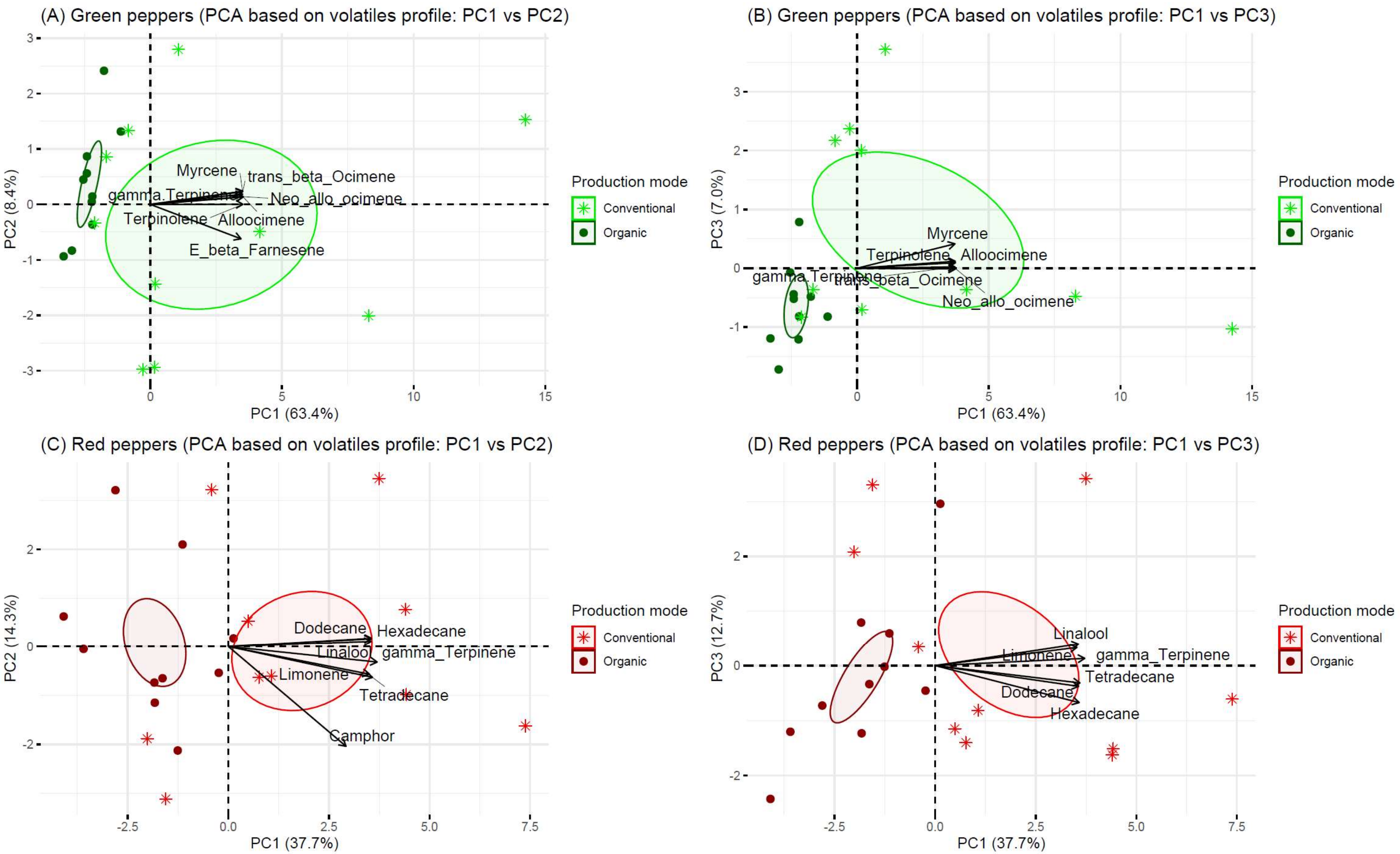

Figure 5.1. Principal component analysis based on the volatile profile of *cv*. Entinas sweet peppers (first three principal component (PC) functions): differentiation of green (A and B) or red (C and D) peppers according to the production mode (conventional versus organic).

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Capítulo 5

Capítulo 6

Sweet peppers discrimination according to agronomic production mode and maturation stage using a chemical-sensory approach and an electronic tongue

Capítulo 6

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Abstract

The demand for organic foods has increased worldwide, in particular due to the association with healthier, more nutritious and tasty products, being a clear trend on sweet peppers' consumption. Thus, this study aimed to evaluate the effects of agronomic production mode (conventional and organic) and maturation stage (associated to green, turning and red colours) on the chemical-sensory attributes of peppers grown in open field. It was found that organic peppers had a better visual/tactile aspect (greater firmness and more intense colours) but lower chemical quality (lower titratable acidity and total soluble solids). On the other hand, red peppers (higher maturation stage) had lower visual-tactile quality but higher chemical quality. From sensory analysis, conventional peppers had better overall aspect, colour intensity-homogeneity and brightness. Then again, the maturation stage of peppers mostly influenced the sensory visual attributes, being turning colour peppers the less appreciated, although organic red peppers were less succulent and had a lower global quality. Even so, the chemical-sensory parameters could be used to discriminate peppers taking into account the agronomic production mode and the maturation stage/colour (79±12% of correct classifications for the repeated K-fold cross-validation procedure). However, a trained sensory panel is required, which can be a major drawback considering their scarcity. This limitation was successfully overcome by using a potentiometric electronic tongue, which allowed discriminating the peppers with a higher predictive sensitivity (85±9%), showing that this device could be used as an accurate taste sensor for the qualitative analysis of sweet peppers.

Keywords: Sweet peppers; Organic/conventional production; Maturation stage; Electronic tongue, Chemometrics

Capítulo 6

6.1. Introduction

Sweet peppers (*Capsicum annuum* L.) belong to the Solanaceae family and are cultivated worldwide, being a highly consumed food due to their attractive colour, pungency, succulence and typical aroma (Eggink et al., 2012). Fresh and processed fruits of different varieties have different colours, from green and white (unripe fruits) to yellow, orange and red (ripe fruits), corresponding to distinct stages of maturation; different sweet and spicy levels; as well as, different shapes, from large to thin fruits (Baenas, et al., 2019). Sweet peppers are commonly produced under conventional or organic farming modes, being the latter based on sustainable and environmentally practices, where the use of pesticides and synthetic fertilizers is forbidden. An increasing worldwide demand for organic products has been observed, mainly due to the consumers growing concerns with health and environmental issues (Massey et al., 2018; Willer & Lernoud, 2018; Denver et al., 2019). The quality of sweet peppers as well as of other fruits and vegetables can be established taking into account physical, chemical and sensory attributes such as appearance, flavour, texture and nutritional value (Rocha et al., 2013). Several researches have focused on peppers' quality characteristics and their evolution during storage or due to different agronomic treatments. The chemical composition, namely volatile and/or non-volatile components and the antioxidant capacity of cultivated or wild species has been highlighted and used for variety identification, geographical origin assessment and/or postharvest chemical/sensory evaluation (Eggink et al., 2012; Barzegar et al., 2018; Caruso et al., 2020; Korkmaz et al., 2020). Although the chemical characterization of a food matrix is of utmost relevance, the flavour (i.e., the overall sensation provided by the interaction of taste, aroma, mouth fell, sight and sound) plays a key role on the consumer's preference, and so, became a main critical quality parameter in pepper production (Eggink et al., 2012). The flavour characteristics are highly dependent on the fruit variety but also on the growing conditions/production modes (e.g., conventional or organic practices) (Hallmann et al., 2019) and on the fruit's maturation stage (Cisternas-Jamet et al., 2020). Despite the relevance of flavour, specific research addressing sweet pepper sensory analysis is scarce (Eggink et al., 2012). Eggink et al. (2012) showed that individual taste attributes of sweet peppers could be linked to volatile and non-volatile compounds of peppers. However, sensory analysis performed by trained panellists is a time consuming and expensive task. Furthermore, the low number of samples that may be evaluate per day, lack of reference standards, the intrinsic human subjective found even for trained panellists, together with the scarcity of trained sensory panels for a specific food matrix, makes sensory analysis a hard task (Eckert et al., 2013).

Thus, emerging sensor-based electrochemical devices have been proposed for sensory analysis, aiming to minimize or even overcoming the abovementioned drawbacks. In this context, it has been highlighted the possibility of using potentiometric and voltammetric electronic tongues (E-tongues) as taste sensors for assessing basic tastes (e.g., acid, pungent, salty, sweet and umami sensations) as well as positive attributes (e.g., bitter, fruity and green sensations) and negative attributes (e.g., rancid, wine-vinegary, musty, fusty, zapateria, butyric, and putrid sensations) of different foodstuffs (Rodrigues et al., 2016; Marx et al., 2017; Borges et al., 2018; Harzalli et al., 2018; Veloso et al., 2018).

This work intended to evaluate the effects of the production mode (i.e., conventional versus organic farming practices) and of the maturation stage (linked to the green, turning colour and red peppers) on physicochemical and sensory attributes of sweet peppers. Also, it was intended to evaluate the possibility of applying a potentiometric E-tongue comprising lipid-polymeric sensor membranes, coupled with chemometric tools for correctly classifying the sweet peppers according to the agronomic production mode/maturation stage. Finally, it was aimed to compare the E-tongue classification performance with that achieved based on the assessment performed by trained panellists.

6.2. Material and methods

6.2.1. Sweet peppers production mode and sampling

Two producers of sweet peppers (*Capsicum annuum* L.), located in the central region of Portugal (Coimbra), were selected. One of the producers followed the organic production European Commission guidelines (Council Regulation (EC) nº 834/2007 of 28 June 2007) whereas the other followed conventional agriculture without any limitations on the use of pesticides. In both fields, the soil had similar acidity, with pH (H_2O) 6.0-6.4, organic matter around 1.5-2.0% and similar physical and chemical characteristics. Sweet pepper seedlings, from Entinas variety, were put in the soil in the last week of May 2018 and were grown under open field conditions. In both fields, a drip irrigation system was installed and the nutritional requirements were supplied by horse manure, in the organic field, and by chemical fertilizers, in the conventional one. In the middle of September 2018, from each producer (organic and conventional) sweet peppers were harvested at three different (increasing) maturation stages (corresponding to green, turning colour and red sweet peppers, respectively). Altogether, five independent batches, of about 2 kg each, of each agronomic production mode and maturation stage were collected, totalizing 30 independent samples. Sweet peppers were then washed, cleaned, dried, and further stored under refrigeration (~4ºC) until analysis.

6.2.2. Sweet peppers chemical, colour and sensory profiles

The sweet peppers collected were subjected to different physicochemical analysis, including texture, colour, titratable acidity (TA), pH and total soluble solids (TSS), using conventional analytical techniques.

The maximum penetration force (N) was evaluated with a HD plus texture analyser (Stable Microsystems, Godalming, UK). The evaluation was made by penetration with a 2 mm cylinder probe, with a 5.0 kg (50 N) charge cell, and a test speed of 1.0 mm/s and 10 mm length. The colour was evaluated using a colorimeter Minolta CR-200b (Osaka, Japan), using the CIELAB scale, namely: L*, a* and b* coordinates, where L* varies between 0 (black) and 100 (white), the chromatic a* axis extends from green (-a*) to red (+a*), and the chromatic b* axis extends from blue (-b*) to yellow (+b*). The evaluations were made in three fruits per sample and for each fruit in four points of the epidermis, with an 8 mm reading aperture, diffuse lighting and an observation angle of 0º under artificial daylight (CIE D65 standard illuminant), being calculated the CIELAB colour coordinates. The titratable acidity (TA) was determined by titrimetric analysis, consisting of a titration with a NaOH solution (0.10 mol/L). Approximately, 10 g of each sample (previously ground) was mixed with 50 mL of water and put on heating under reflux for 30 min. Then, the resultant solution was transferred to a glass balloon of 100 ml and after filtration a precise volume (20 mL) was transferred to a beaker with a stirrer. Then, the pH of the solution was monitored continuously in order to obtain the titration curve. The pH at the equivalence point was established as 8.1, as indicated in the Portuguese regulation (NP-1421, 1977). The values were expressed on mg citric acid /100 g fresh weight (fw). pH values were evaluated using a Crison-Micro pH 2002 (Crison, Barcelona, Spain) potentiometer. The solution obtained for the acidity determination (after filtration) was also used to measure total soluble solids (TSS) contents (ºBrix), at 20 ºC, in an ATAGO refractometer (Saitama, Japan).

Sensory analysis of peppers was performed by a 8 trained sensory panel from the National Institute for Agricultural and Veterinary Research (INIAV, Portugal). The attributes evaluated by the panellists according to the guidelines of the International Organization for Standardization (ISO 11036: 1994; ISO 4121: 2003; ISO 13299: 2016), with some adaptations. From each batch, three peppers pieces of 2 cm^2 were evaluated considering the aspect, intensity and homogeneity of colour, brightness, aroma, sweet, bitter, acid, pungent, taste, hardness, fibrousness, crustiness, succulence and global quality. All of these parameters were assessed using a continuous unstructured scale from 1 (absence of sensation) to 11 (maximum intensity).

6.2.3. E-tongue analysis

6.2.3.1. Apparatus.

A lab-made potentiometric E-tongue multi-sensor device comprising two cylindrical arrays has been used (Figure 6.1). Each array contained 20 lipid polymeric cross-sensitive sensor membranes (40 sensors in total), which composition (lipid additive, 3%; plasticizer, 32%; and, polyvinyl chloride, 65%) has been previously described (Harzalli et al., 2018) although used sensor membranes had a higher contact surface (~25 mm^2) and thickness, allowing a better signal stability and the detection performance. The membranes were connected to a multiplexer Agilent Data Acquisition Switch Unit (model 34970A) controlled by the Agilent BenchLink Data Logger. Each potentiometric assay took 5 min and allowed recording the potentiometric signals of all membranes generated due to electrostatic and/or hydrophobic interactions (Kobayashi et al., 2010). An Ag/AgCl rdouble-junction glass reference electrode (Crison, 5241) was used. The same sensor coding used in previous works was adopted: each sensor was identified with a letter S (for sensor) followed by the number of the array (1 or 2) and the number of the membrane (1 to 20, corresponding to different combinations of plasticizers and additives).

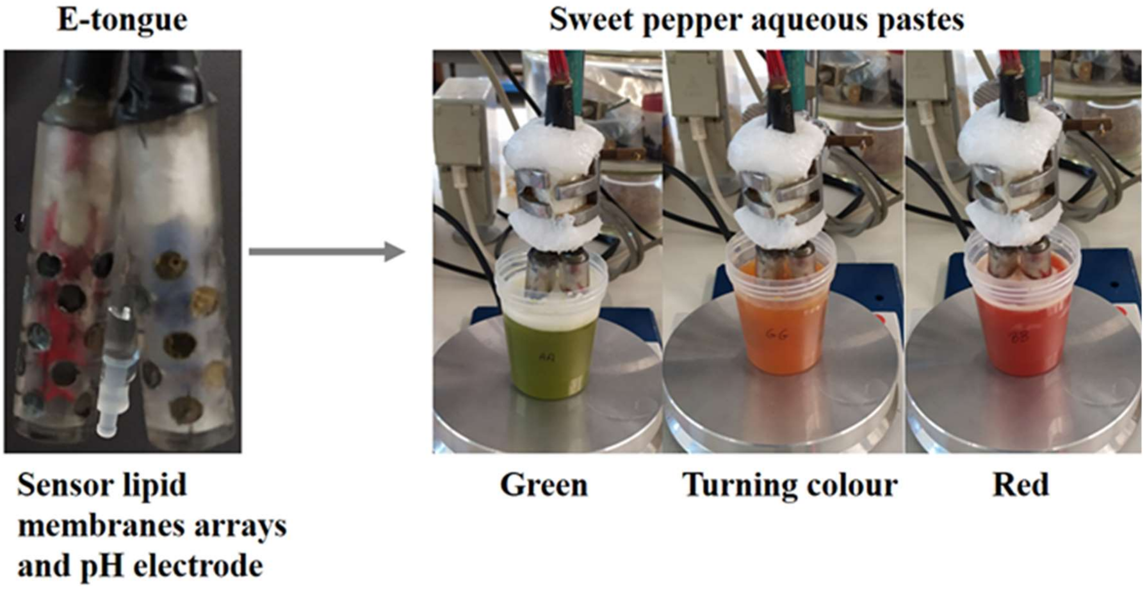

Figure 6.1 Lab-made E-tongue comprising two cylindrical arrays with lipid-polymeric 518 membranes, used as a taste sensor device for the potentiometric analysis of sweet peppers.

6.2.3.2. E-tongue analysis: sweet pepper sample preparation and potentiometric assays.

For the potentiometric assays, sweet peppers were processed following the procedure described by Marx et al. (2017) with some modifications. From each sweet pepper sample, two rectangular portions of about 8 cm × 6 cm were cut (~25 g per portion) and separately mashed using a Moulinex knife chopper. For each replicate, 20 g of mashed sweet pepper were placed in a plastic cup (100 mL), being then diluted with 60 mL of deionized water and agitated during 1 min. The aqueous paste was left in the fridge overnight (~4ºC), being the potentiometric analysis carried out during the next 24 hours, after letting them reach the ambient temperature (~20ºC) and after a smooth agitation (~1 min). So, for each sweet pepper replicate, two electrochemical assays were performed, with a third assay carried out if the potentiometric signal of any of the 40 sensors showed a coefficient of variation greater than 20% (Veloso et al., 2018). Afterwards, for data split (random establishment of training and internal-validation sets) and modelling purposes, only one electrochemical "average" signal profile per sample (i.e., per sweet pepper from the independent quintuplicate assays) was used, avoiding that data from duplicate assays of the same sweet pepper could be included into both training and validation sets (Rodrigues et al., 2016).

6.3. Statistical analysis

The significant statistical effects of the agronomic production mode as well as of the maturation stages on the chemical, colour and sensory profiles of sweet peppers, was evaluated using the t-Student test and the one-way ANOVA (followed by the post-hoc multi-comparison Tukey's test if a significant statistical effect was found, i.e., P-value < 0.05), respectively.

Principal component analysis (PCA) and linear discriminant analysis (LDA) coupled with the meta-heuristic simulated annealing (SA) variable selection algorithm were used to evaluate the unsupervised and supervised classification power of the chemical-sensory data as well as of the E-tongue signal data. The LDA-SA aimed to identify the most non-redundant discriminative sub-set of independent variables by minimizing possible noise effects (Cadima et al., 2004). The predictive performances of the LDA-SA models were checked using the leave-one-out (LOO) and the repeated K-fold cross-validation (CV) variants . For the latter the number of folds (K) was set equal to 4 and the number of repetitions equal to 10. All variables were scaled and centered before modelling to normalize the weight of each one on the final multivariate linear classification model. The unsupervised and supervised classification performances were qualitatively assessed using 3D plots of the principal or discriminant components and, for the LDA-SA models by calculating the sensitivity values (i.e., the percentage of correctly classified samples). The statistical analysis was performed using the Subselect (Cadima et al., 2004) and MASS (Venables, & Ripley, 2002) packages of the open source statistical program R (version 2.15.1), at a 5% significance level.

6.4. Results

6.4.1 Chemical, colour and sensory profiles of sweet peppers

The sweet peppers' physicochemical and sensory data, produced under agronomic conventional or organic modes and harvested at three increasing maturation stages (green, turning colour and red, respectively) are shown in Tables 6.1 and 6.2, respectively. The values determined regarding the fruit quality parameters (force, TSS, TA, pH and CIELAB colour scale) are in agreement with the majority of the vast data reported in literature for sweet peppers. Although it can be stated that values are usually of the same order of magnitude, it should be noticed that quality parameters are highly dependent on the fruit variety, maturation stage, agronomic production practices (e.g., irrigation deficit conditions, soilless media composition, radiative environment, field crops conditions, among other) and geographical origin and so, in some specific cases, differences arisen (Eggink et al., 2012; Selahle et al., 2015; Aslani et al., 2016; Bayogan et al., 2017; Barzegar et al., 2018; Gajc-Wolska et al., 2018;

Jimenez-Garcia et al., 2018; Almeida Alves et al., 2019; Ibrahim & Abdulah, 2019; Ibrahim et al., 2018; Neocleous & Nikolan, 2019; Caruso et al., 2020).

Table 6.1. Physicochemical experimental composition (mean value ± standard deviation) and statistical differences for sweet peppers with different maturation stages (green, turning colour and red corresponding to increasing maturation, respectively) and produced under different agronomic production mode (conventional and organic farming practices)

Parameter		Production mode	Sweet pepper colour (*i.e.*, maturation stage)			
			Green	*Turning colour*	*Red*	*P*-value[i]
Force (N)		*Organic*	**12.1±2.3**[a] (8.2-16.7)	**11.1±1.4**[a] (8.4-13.4)	**9.2±1.4**[b] (7.3-12.3)	***< 0.0001***
		Conventional	**7.9±0.9**[b] (6.5-10.0)	**9.8±1.2**[a] (7.2-11.7)	**7.9±1.2**[b] (6.1-10.0)	***< 0.0001***
		P-value[ii]	***< 0.0001***	***0.0001***	***0.0002***	
CIELAB colour scale	**L***	*Organic*	**37.4±1.6**[a] (34.2-41.3)	**37.6±3.3**[a] (32.0-43.9)	**34.7±2.2**[b] (31.-40.5)	***0.0006***
		Conventional	**36.0±2.7**[a] (31.9-39.8)	**33.3±2.3**[b] (29.0-37.0)	**31.0±1.6**[c] (27.7-33.7)	***< 0.0001***
		P-value[ii]	0.0551	***< 0.0001***	***< 0.0001***	
	a*	*Organic*	**-11.1±1.2**[c] (-13.9-(-)9.4)	**5.3±6.2**[b] (-3.0–17.7)	**22.3±3.8**[a] (15.1-28.3)	***< 0.0001***
		Conventional	**-12.4±2.1**[c] (-15.5-(-)8.9)	**9.1±11.0**[b] (-11.7-29.1)	**19.4±4.0**[a] (12.0-26.4)	***< 0.0001***
		P-value[ii]	***0.0194***	0.1936	***0.0265***	
	b*	*Organic*	**14.7±2.9**[b] (9.0-22.5)	**17.8±4.2**[a] (11.9-27.0)	**15.4±2.7**[a,b] (11.9-21.7)	***0.0120***
		Conventional	**17.0±4.2**[a] (10.6-22.5)	**13.3±2.8**[b] (9.2-19.3)	**11.6±2.3**[b] (8.9-19.0)	***< 0.0001***
		P-value[ii]	0.0551	***0.0003***	***< 0.0001***	
Titratable acidity (TA, mg citric acid/100 g fw)		*Organic*	**63±4**[b] (56-69)	**152±19**[a] (114-175)	**162±16**[a] (145-189)	***< 0.0001***
		Conventional	**67±9**[b] (55-83)	**168±13**[a] (147-189)	**171±18**[a] (151-202)	***< 0.0001***
		P-value[ii]	0.1404	***0.0155***	0.1576	
pH		*Organic*	**6.2±0.1**[a] (6.1-6.3)	**5.0±0.1**[b] (4.9-5.2)	**5.0±0.1**[b] (4.7-5.1)	***< 0.0001***
		Conventional	**6.0±0.5**[a] (5.9-6.6)	**5.5±0.4**[b] (5.1-5.6)	**5.2±0.1**[c] (5.0-5.3)	***< 0.0001***
		P-value[ii]	0.2483	***< 0.0001***	***0.0001***	
Total soluble solids (TSS, ºBrix)		*Organic*	**3.8±0.3**[b] (3.2-4.4)	**5.8±0.8**[a] (4.6-7.0)	**5.8±0.7**[a] (4.4-7.2)	***< 0.0001***
		Conventional	**4.4±0.5**[b] (3.9-5.7)	**7.8±0.7**[a] (6.2-8.9)	**7.6±0.5**[a] (6.9-8.3)	***< 0.0001***
		P-value[ii]	***0.0003***	***< 0.0001***	***< 0.0001***	

On the contrary, a scarce number of works described the assessment of intensities of sensory attributes by panellists (Eggink et al., 2012; Bayogan et al., 2017; Jimenez-Garcia et al., 2018) and, in general, the information reported cannot be easily used for comparison due to the different attributes evaluated and the different intensity scales used by the different research teams. Even so, for comparable/similar assessed sensations (e.g., acid, aroma, crunchy, hardness, juicy, pungent, salty, sweet, visual aspect), the intensities perceived, in this work, by the trained panellists are in slight agreement with those reported in the literature (Eggink et al., 2012; Bayogan et al., 2017; Jimenez-Garcia et al., 2018). Tables 6.1 and 6.2 show that the agronomic production mode and the maturation stage had, in general, a significant statistical effect (P-value < 0.05) on sweet peppers' physicochemical parameters and sensory attributes.

For the evaluated physicochemical parameters, and independently of the peppers' maturation stage (i.e., colour) it could be stated that organic fruits had greater firmness (evaluated based on the force data) and higher values of CIELAB colour coordinates (L*, a* and b*) in comparison with conventional peppers (in general, P-values < 0.05 for t-Student's test, Table 1), which constituted a visual/tactile advantage for the former mode. An opposite inference could be observed for the other parameters, showing the organic fruits lower pH, TA and TSS values than conventional fruits (in general, P-values < 0.05 for t-Student's test, Table 1), showing that the latter mode would allow producing peppers richer in organic acids and with higher sugar contents, and thus with a higher chemical quality. In general, the results also pointed out that, regardless the agronomic production mode, increasing peppers' maturation stage (i.e., red > turning colour > green) would lead to a significantly decrease trend of the fruits' firmness, the L* and b* coordinates values as well as of the pH values and to a significantly increase of the fruits' a* coordinate value, TA and TSS values (P-value < 0.05 for one-way ANOVA and post hoc Tukey's HSD test). So, peppers at higher maturation stage would show a lower visual and tactile quality but higher chemical quality (i.e., greater levels of organic acids and sugar contents.

In which concerns the sensory analysis, the trained panellists evaluated attributes related to the visual aspect, olfactory and taste sensations. Table 6.2 shows that, independently of the peppers' maturation stage, the production mode only had a clear significant statistical influence on the visual aspect parameters. In fact, peppers produced following the conventional mode were scored with significantly higher (P-values < 0.05 for t-Student's test) values regarding the overall aspect, colour intensity and homogeneity as well as brightness compared to organic peppers. On the other hand, and generally, the production mode did not had a significant effect on olfactory and gustatory attributes (i,e., aroma, acid, sweet, bitter, pungent, taste, hardness, fibrousness, crustiness, succulence) neither on the peppers' global quality. Regarding the maturation stage (i.e., fruit's colour) effect on the sensory sensations evaluated, independently of the production mode, once again it could be observed that it was more significant on the visual aspect parameters (with the exception of brightness), being evident that turning colour peppers had significant lower scores regarding the overall aspect, colour intensity and homogeneity, than green and red peppers (P-value < 0.05 for one-way ANOVA and post hoc Tukey's HSD test). In which concerns the aroma and some of the basic tastes (sweet, bitter, pungent and taste), although some significant differences could be found between peppers harvested at different maturation stages (i.e., different colours) and produced following different agronomic practices, the observed differences were punctual and did not have a clear trend.

Also, no significant maturation stage effect was observed for hardness, fibrousness, and crustiness, being found a significant decrease of the succulence intensity and the global quality with the increase of the maturation stage but only for organic production, indicating that red peppers were the less appreciated ones and that he organic mode could be more prone to fruit's maturation influence. Finally, it should be pointed out the effects of the agronomic production mode on the quality of agricultural products and health benefits are not consensual. Some studies evidenced that organic fruits/vegetables are usually more aromatic, with more intense flavour, better sensory characteristics and healthier composition, although the positive impact on human health is not clear (Brantsaeter et al., 2017). In the present work, in general, TA was higher in conventional peppers, which could be related to a higher content in organic acids. Also the pH values were lower in organic peppers, although the values were similar to those found for conventional mode, and so, this observation could be tentatively attributed to slight differences in soil characteristics. The TSS values were higher in conventional peppers, which may indicate that the plants grown under conventional mode were healthier from a nutritional point of view, allowing obtaining peppers with higher sugar levels and consequently with a better quality. In which concerns the fruit's maturation effect (i.e., pepper's colour) the results showed a marked significant effect on both physicochemical and sensory characteristics. Within the mode of production, differences were observed with fruit maturation, from green to red. As well, independently of the fruit's maturation stage, the production mode also had a significant impact. The results seem to indicate that in terms of global quality, and under the experimental conditions of this work, peppers produced following agronomic conventional mode had, in general, better physicochemical characteristics and a higher global quality.

Table 6.2. Sensory attributes (mean value ± standard deviation) perceived by trained panellists and statistical differences for sweet peppers with different maturation stages (green, turning colour and red corresponding to increasing maturation, respectively) and produced under different agronomic production mode (conventional and organic farming practices).

Sensory attribute[i]	Production mode	Sweet pepper colour (*i.e.*, maturation stage)			
		Green	*Turning colour*	*Red*	*P*-value[ii]
Aspect	*Organic*	**9.0±1.3**^{a}	**8.0±1.8**^{b}	**8.2±1.5**^{a,b}	***0.0094***
		(6.0-11.0)	(4.4-10.4)	(4.2-10.4)	
	Conventional	**9.5±1.0**^{a}	**8.4±1.4**^{b}	**9.3±1.2**^{a}	***0.0003***
		(6.8-10.8)	(4.5-10.8)	(7.0-11.0)	
	P-value[iii]	0.0644	0.1897	***0.0004***	
Colour intensity	*Organic*	**7.9±1.7**^{a}	**6.4±1.8**^{b}	**7.9±1.9**^{a}	***0.0002***
		(4.3-10.1)	(3.2-10.3)	(4.2-10.6)	
	Conventional	**8.7±1.5**^{a,b}	**8.1±1.3**^{b}	**9.2±1.3**^{a}	***0.0024***
		(5.0-10.8)	(5.3-10.2)	(6.0-11.0)	
	P-value[iii]	***0.0407***	***< 0.0001***	***0.0009***	
Colour homogeneity	*Organic*	**9.0±1.1**^{a}	**4.8±2.2**^{b}	**8.4±1.7**^{a}	***< 0.0001***
		(5.5-10.5)	(1.5-9.6)	(2.6-10.3)	
	Conventional	**9.6±1.1**^{a}	**6.2±2.5**^{b}	**9.5±1.3**^{a}	***< 0.0001***
		(7.1-11.0)	(1.9-10.2)	(5.2-11.0)	
	P-value[iii]	***0.0254***	***0.0094***	***0.0008***	
Brightness	*Organic*	**7.8±1.3**	**7.3±1.4**	**7.5±1.6**	0.3592
		(3.7-9.8)	(3.1-9.4)	(3.6-9.7)	
	Conventional	**8.2±1.4**	**7.6±1.4**	**8.1±1.3**	0.1763
		(6.1-10.5)	(4.9-9.8)	(5.3-10.2)	
	P-value[iii]	0.1666	0.2620	0.0819	
Aroma	*Organic*	**7.0±1.8**^{a}	**6.3±1.6**^{a,b}	**5.4±2.0**^{b}	***0.0004***
		(2.8-10.1)	(3.3-10.0)	(2.4-9.7)	
	Conventional	**7.4±1.3**	**6.9±1.7**	**7.1±1.7**	0.3267
		(5.5-9.9)	(3.2-10.1)	(3.2-10.0)	
	P-value[iii]	0.2232	0.1106	***< 0.0001***	
Sweet	*Organic*	**4.6±1.8**	**5.1±1.8**	**4.7±1.5**	0.4083
		(1.3-8.4)	(2.3-9.4)	(2.4-8.7)	
	Conventional	**4.5±1.8**^{b}	**5.3±1.5**^{a,b}	**5.5±1.5**^{a}	***0.0144***
		(1.9-10.2)	(2.3-8.3)	(2.5-8.6)	
	P-value[iii]	0.7209	0.5736	***0.0284***	
Bitter	*Organic*	**2.4±1.2**^{a}	**2.0±0.9**^{a,b}	**1.8±0.8**^{b}	***0.0265***
		(1.0-5.2)	(1.0-4.3)	(1.0-4.3)	
	Conventional	**2.5±1.5**	**2.0±1.0**	**2.2±1.2**	0.2177
		(1.0-7.1)	(1.0-5.2)	(1.0-5.6)	
	P-value[iii]	0.7743	0.9138	0.1255	
Acid	*Organic*	**1.8±1.0**	**1.9±0.9**	**1.6±0.8**	0.4196
		(1.0-5.2)	(1.0-3.8)	(1.0-3.5)	
	Conventional	**2.2±1.5**	**1.7±0.9**	**2.0±1.2**	0.2246
		(1.0-6.5)	(1.0-4.1)	(1.0-6.1)	
	P-value[iii]	0.1729	0.4599	0.0638	
Pungent	*Organic*	**1.9±0.9**	**2.0±1.2**	**1.7±0.8**	0.3231
		(1.0-3.8)	(1.0-4.8)	(1.0-3.7)	
	Conventional	**2.7±1.5**^{a}	**2.0±1.1**^{b}	**2.2±1.4**^{a,b}	***0.0482***
		(1.0-6.5)	(1.0-4.8)	(1.0-6.6)	
	P-value[iii]	***0.0045***	0.9464	***0.0481***	
Taste	*Organic*	**6.0±1.8**^{a}	**6.0±1.9**^{a}	**4.9±1.8**^{b}	***0.0107***
		(2.7-8.9)	(1.9-10.0)	(1.7-9.0)	
	Conventional	**7.0±1.6**^{a}	**6.1±1.7**^{b}	**6.8±1.5**^{a,b}	***0.0487***
		(3.3-10.2)	(2.8-9.2)	(3.4-9.2)	
	P-value[iii]	***0.0108***	0.7463	***< 0.0001***	
Hardness	*Organic*	**8.8±1.2**	**8.3±1.2**	**8.5±1.3**	0.3366
		(6.3-10.6)	(5.6-10.0)	(5.4-10.2)	

	Conventional	**8.6±1.4** (5.6-10.4)	**8.5±1.1** (6.4-10.2)	**8.2±1.1** (5.6-9.5)	0.2260
	P-value[iii]	0.5650	0.4396	0.2608	
Fibrousness	*Organic*	**5.6±2.8** (1.1-9.7)	**6.0±2.8** (1.1-9.7)	**6.2±3.0** (1.3-10.5)	0.6603
	Conventional	**5.8±2.5** (1.0-9.0)	**6.0±2.8** (1.1-10.1)	**5.6±2.9** (1.0-9.5)	0.8127
	P-value[iii]	0.6896	0.9968	0.4118	
Crustiness	*Organic*	**8.9±1.4**[a] (5.8-10.6)	**8.4±1.4**[a,b] (5.6-10.6)	**8.1±1.3**[b] (5.5-10.4)	***0.0447***
	Conventional	**8.9±1.3** (4.9-10.4)	**8.5±1.4** (5.3-10.5)	**8.2±1.3** (4.8-10.2)	0.0582
	P-value[iii]	0.9484	0.6759	0.8272	
Succulence	*Organic*	**9.0±1.1**[a] (5.0-10.6)	**8.1±1.4**[b] (5.3-10.6)	**8.1±1.2**[b] (5.4-10.5)	***0.0014***
	Conventional	**8.2±1.4** (5.7-10.3)	**8.3±1.2** (5.0-10.2)	**8.3±1.4** (4.6-10.0)	0.9860
	P-value[iii]	***0.0079***	0.5816	0.5361	
Global quality	*Organic*	**8.0±1.3**[a] (3.9-10.4)	**7.3±1.3**[a] (5.2-10.2)	**6.6±1.4**[b] (3.4-9.6)	**< *0.0001***
	Conventional	**8.0±1.4** (5.6-10.5)	**7.5±1.3** (4.5-10.3)	**7.9±1.3** (5.6-10.7)	0.1966
	P-value[iii]	0.9138	0.5840	**< *0.0001***	

6.4.2. Discrimination of sweet peppers according to production mode and maturation stage

6.4.2.1 PCA and LDA-SA models based on chemical-sensory data

As previously shown, the production mode as well as the maturation stage significantly influenced the chemical parameters and the sensory profile. Thus, the possibility of using the chemical-sensory profile for the unsupervised classification of the sweet peppers according to the 6 levels of production mode/fruit colour (i.e, maturation stage) was evaluated using PCA. Figure 6.2A showed that the 4 chemical parameters together with the 15 sensory attributes allowed a correct unsupervised split of the sweet peppers by production mode/fruit colour, based on the first 3 principal components (1st, 2nd and 3rd PCs explaining 67% of the total variance). Indeed, it was possible to infer that, the chemical-sensory data would primarily allow differentiating the green sweet peppers from the others and, secondly, to discriminate the organic from the conventional production modes. To check this promising unsupervised classification performance, a LDA-SA approach was used. The results showed that it was possible to establish a LDA-SA model based on 11 parameters (3 chemical parameters: force, TSS and TA; and, 8 sensory attributes: aspect, colour intensity, aroma, sweet, acid, taste, crustiness and global quality). The first 3 significant linear discriminant (LD) functions explained 98% of the data variance allowing to correctly classify 100% (Figure 6.2B) of the original grouped data (training) and only 80% of the data for the LOO-CV (internal-validation) procedure. In this latter case, the majority of the misclassification occurred between the organic and conventional turning colour sweet peppers. Taking into account that this CV variant is known as overoptimistic, the predictive performance of the LDA-SA model based on the 11 chemical-sensory parameters was further evaluated using the repeated K-fold-CV procedure (4 folds and 10 repeats). With this more robust technique, which allowed to reserve 25% of the data (at least one sample from each of the 6 groups) for validation purposes, an average predictive sensitivity of 79±12% was achieved, pointing out that, although the referred parameters could be used to discriminate the sweet peppers, it only could be used as a preliminary tool.

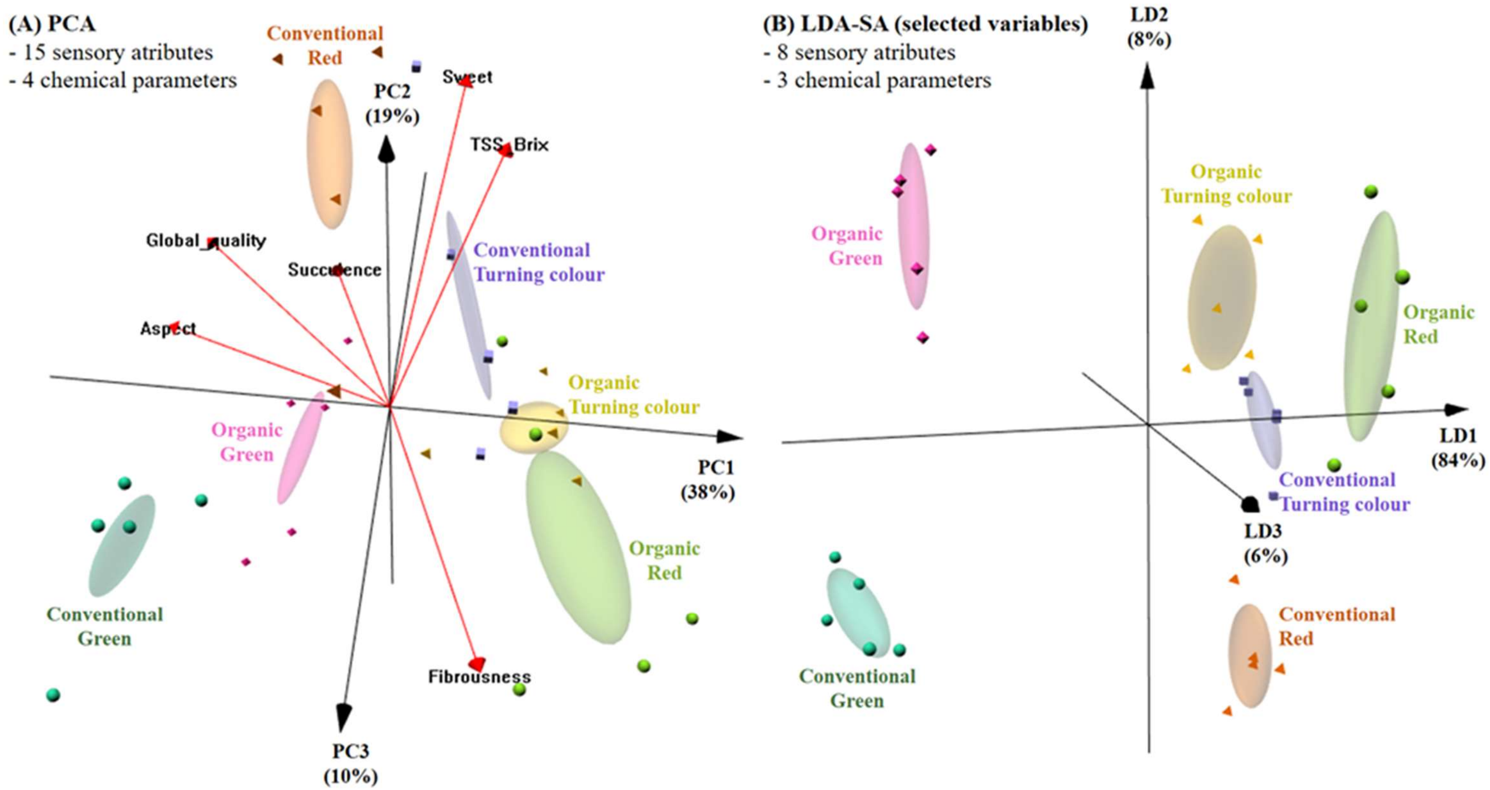

Figure 6.2. Classification of sweet peppers according to the production mode (organic and conventional) and the fruit colour (green, red and turning colour, i.e., different maturation degrees) using chemical and sensory data as discriminator variables**:(A)** 3D-PCA plot of the first 3 PCs based on 4 chemical parameters (force, TSS, pH and TA) and 15 sensory attributes (aspect, colour intensity and homogeneity, brightness, aroma, sweet, bitter, acid, pungent, taste, hardness, fibrosis, crustiness, succulence and global quality); and, **(B)** 3D-LDA plot of the first 3 DFs based on 3 chemical parameters (force, TSS and TA) and 8 sensory attributes (aspect, colour intensity, aroma, sweet, acid, taste, crustiness and global quality), selected using the SA algorithm.

6.4.2.2 PCA and LDA-SA models based on E-tongue signal profiles

E-tongues, namely potentiometric taste-sensors comprising non-specific and cross-sensitive lipid sensor membranes, have been developed by the research team and successfully applied for qualitative and quantitative analysis of foods, like, for example, soft beverages (Dias et al., 2014), still and sparkling mineral waters (Dias et al., 2016), honey (Sousa et al., 2014), table olives (Marx et al., 2017) and olive oil (Borges et al., 2018; Harzalli et al., 2018; Veloso et al., 2018). In this work, for the first time, the performance of a potentiometric E-tongue for discriminating sweet peppers according to the production mode and fruit colour (i.e., maturation stage) was investigated. First, PCA was carried out using the signal profiles recorded by the 40 sensors of the E-tongue, during the potentiometric analysis of the aqueous sweet pepper pastes. The results (Figure 3.3A) showed that sweet pepper potentiometric fingerprints could be used for the unsupervised differentiation of green sweet peppers (organic or conventional) from the red and turning colour sweet peppers, although for these latter a high degree of samples overlapping was observed. The predictive classification capability of the E-tongue was deeply assessed using the LDA-SA approach. An E-tongue-LDA-SA model could be established based on the potentiometric profiles gathered by a selected sub-set of 13 sensors (S1:5, S1:9, S1:10, S1:12, S1:14, S1:18, S2:3, S2:4, S2:8, S2:10, S2:13, S2:16 and S2:18), explaining the first 3 discriminant functions 99.9% of the data variance. The multivariate linear model allowed 100% of correct classifications for the original grouped data (Figure 3.3B) and 90% for the LOO-CV (turning colour sweet peppers from conventional production showed the greatest number of misclassified samples), showing a better accuracy compared to the LDA-SA model previously established based on 11 chemical-sensory parameters. The predictive classification performance was further checked according to the repeated K-fold-CV internal-validation procedure (4 folds × 10 repeats) resulting in an average sensitivity of 85±9%. This result confirmed the superiority of the E-tongue discrimination model, showing that it could be used as a practical and more accurate tool for a preliminary recognition of the sweet pepper production mode and maturation stage. This achievement also pointed out that the potentiometric E-tongue could be used as an indirect taste sensor, since by allowing assessing the fruit colour it also indirectly evaluates the evolution trends of the sweet, bitter, pungent and acid taste sensations. Although these attributes can be perceived by trained panellists, the availability of sensory panels is scarce. Moreover, the sensory evaluation by panellists may pose several limitations, such as panellists score subjectivity, analysis cost and low number of samples that can be evaluated per day, which may be minimized or partially overcome using this type of E-tongue as a prospective routine analytical tool.

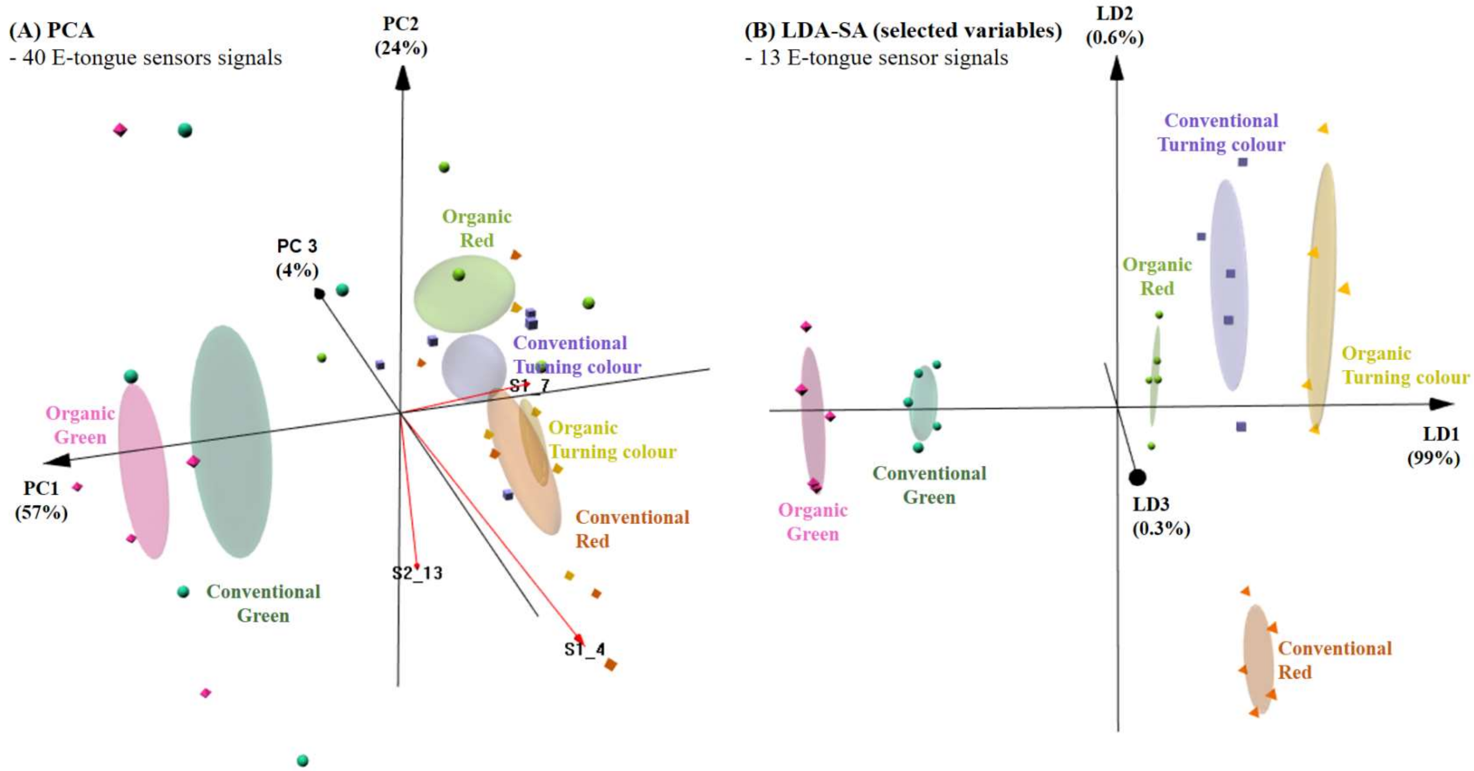

Figure 6.3. Classification of sweet peppers according to the production mode (organic and conventional) and the fruit colour (green, red and turning colour, i.e., different maturation degrees) using the E-tongue signal profiles as discriminator variables: (A) 3D-PCA plot of the first 3 PCs based on the potentiometric signals gathered by the 40 E-tongue sensors (S1:1 to S1:20, S2:1 to S2:20); and, (B) 3D-LDA plot of the first 3 DFs based on 13 E tongue sensors (S1:5, S1:9, S1:10, S1:12, S1:14, S1:18, S2:3, S2:4, S2:8, S2:10, S2:13, S2:16 and S2:18), selected using the SA algorithm.

6.5. Conclusions

The study carried out allowed confirming that the physicochemical composition and sensory attributes of sweet peppers are highly dependent on the agronomic production mode and on the maturation stage. For the conditions evaluated and the variety Entinas studied, it could be concluded that, in general, sweet peppers produced under the organic production mode had a better visual and tactile quality and lower chemical quality than fruits produced using agronomic conventional practices. On the other hand, the production mode and the fruit's maturation stage did not had a significant effect on olfactory and gustatory attributes neither on the peppers' global quality, although a significant effect was found on the visual aspect sensory attributes. Moreover, the work pointed out that the physicochemical and sensory data could be used to satisfactorily classify the studied peppers according to the production mode-maturation stage. Finally, it was also demonstrated, for the first time, that a potentiometric electronic tongue could be successfully used as a preliminary taster sensor for correctly discriminating sweet peppers according to the conventional or organic production mode and taking into account the fruits' colour. This finding allows foreseen the electronic tongue as a possible complementary sensory analysis tool that could be applied for overcoming the known shortcomings of the sensory panels, namely their scarcity and reduced number of samples that can be daily evaluated.

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Capítulo 6

Capítulo 7

Conclusões finais

Capítulo 7

7. Conclusões finais

A produção de alimentos assume, nos dias de hoje, uma dimensão que vai muito além da quantidade produzida. Para o consumidor, a garantia de uma produção ambiental e socialmente sustentável são aspetos com um peso elevado na decisão de compra, para além das caraterísticas qualitativas e nutricionais intrínsecas dos próprios alimentos.

Na atualidade, estão regulamentados dois modos de produção agrícola, o modo de produção biológico, e o modo de produção integrada, também designado de convencional ao longo deste trabalho. Em ambos se procura produzir alimentos de qualidade, transacionáveis a um preço justo e acessíveis para os consumidores, utilizando técnicas de produção sustentáveis a longo prazo, que sejam capazes de assegurar rendimento para os agricultores. No entanto existem diferenças em ambos os modos, sendo a mais notória a utilização de produtos químicos de síntese, como fertilizantes, pesticidas e promotores de crescimentos, permitidos em produção integrada, mas não admitidos no modo de produção biológico.

No ato de compra muitos dos consumidores optam por alimentos produzidos em modo de produção biológico por considerarem que estes têm melhor qualidade, ainda que não do ponto de vista visual, mas sobretudo dos pontos de vista sensorial e composicional, com grande relevância para a existência de maior teor de bioativos. Contudo, a literatura da especialidade não é conclusiva relativamente a este aspeto, existindo trabalhos que demonstram que em condições agroambientais semelhantes os produtos originários do modo de produção biológico apresentam melhores caraterísticas qualitativas, enquanto outros estudos apontam o sentido inverso, com melhores caraterísticas atribuídas aos produtos de produção integrada, existindo ainda um considerável número de trabalhos que é inconclusivo nestes aspetos.

Neste sentido, esta tese de doutoramento pretendeu contribuir para o esclarecimento de alguns dos aspetos referidos, nomeadamente no que respeita à qualidade de produtos obtidos em modo de produção biológico em comparação com o modo de produção convencional, ou em produção integrada. Nesse sentido o estudo incidiu sobre o pimento doce, *C. annuum* variedade Entinas, tendo-se obtido numa primeira fase informação acerca de aspetos da cultura em modo de produção biológico na região de Coimbra, procedendo-se depois à comparação de pimentos produzidos em modo de produção biológico e convencional, estudados em dois estados de maturação distintos, verde e vermelho.

De seguida apresentam-se as principais conclusões respeitantes aos diferentes capítulos.

Capítulo 2:

- Nas condições agroecológicas de Coimbra, a cultura do pimento ao ar livre, em modo de produção biológico, desenvolve-se normalmente e atingem-se produtividades consideradas elevadas, na ordem das 28 $t.ha^{-1}$, ainda que consideravelmente inferiores aos valores registados nas estatísticas agrícolas para o nosso País, mas superiores à média mundial;

Capítulo 3:

- No que respeita à composição mineral, em macro e micro nutrientes, foi possível observar uma clara separação entre a constituição dos pimentos da produção biológica e da produção convencional.
- Em ambos os modos de produção e em ambos os estados de maturação a concentração em macronutrientes apresentou, por ordem decrescente, a seguinte sequência: potássio (K) > fósforo (P) > cálcio (Ca) > enxofre (S). O potássio é o macronutriente em maior concentração no pimento.
- Em ambos os modos de produção e estados de maturação a concentração em micronutriente apresentou, por ordem decrescente, a seguinte sequência: cloro (Cl) > ferro (Fe) > zinco (Zn) > manganês (Mn) > cobre (Cu). O cobre é o micronutriente presente em menor concentração no pimento.
- Os pimentos do modo de produção biológico, independentemente do estado de maturação, tiveram maiores concentrações de potássio (K), fósforo (P), cálcio (Ca) e cobre (Cu).
- Os teores de cloro (Cl), ferro (Fe) e enxofre (S) foram superiores nos pimentos produzidos em modo de produção biológico, enquanto os de manganês (Mn) e de zinco (Zn) foram mais altos nos de produção convencional.
- Os pimentos da produção convencional exibiram maiores teores de gordura comparativamente aos biológicos e independentemente do estado de maturação.

Capítulo 4:

- Foram identificados nove compostos fenólicos ácido cafeico, ácido clorogénico, ácido m-cumárico, ácido o-cumárico, luteolina-7-O-glucósido, miricetina, resveratrol, rutina e quercetina-3-O-rhamnosideo.
- Os pimentos verdes foram mais ricos em compostos fenólicos totais e expressaram maior atividade antioxidante comparativamente aos vermelhos, apesar de alguns dos compostos identificados estarem mais presentes nos pimentos vermelhos.
- De uma forma geral os pimentos do modo convencional apresentaram maiores teores em compostos fenólicos e atividade antioxidante.

- A utilização de ferramentas estatísticas permitiu separar de forma muito satisfatória as amostras de pimentos de acordo com o seu grau de maturação e o modo de produção o que indica que estes compostos podem ser utilizados como biomarcadores.

Capítulo 5:

- Foram identificados e quantificados 48 compostos voláteis pertencentes às classes: dos aldeídos, cetonas, alcóois, esteres, sesquiterpenos, terpenos, alcanos e hidrocarbonetos.
- No que respeita aos modos de produção foram identificados 43 compostos voláteis nos pimentos provenientes do modo de produção convencional e 34 compostos nos pimentos oriundos do modo de produção biológico.
- Relativamente aos compostos identificados nos dois estados de maturação (verde e vermelho) verificou-se que os pimentos verdes convencionais apresentaram um total de 37 compostos e os de produção biológica 30; nos pimentos vermelhos convencionais foram identificados 30 compostos e nos de produção biológica 25 compostos.
- Em ambos os modos de produção os pimentos verdes apresentaram maiores teores de voláteis que os pimentos vermelhos.

Capítulo 6:

- Os pimentos produzidos no modo de produção biológico apresentaram melhor qualidade visual e tátil e menor qualidade química do que os pimentos produzidos pelas práticas agronómicas convencionais quando avaliados por um painel sensorial.
- A aplicação de uma língua eletrónica potenciométrica permitiu discriminar corretamente pimentos de acordo com o modo de produção convencional ou modo de produção biológico e também estado de maturação dos frutos.
- A utilização de língua pode ser uma ferramenta de grande utilidade na análise de um elevado número de amostras e na complementaridade da análise sensorial ultrapassando as conhecidas deficiências dos painéis sensoriais, nomeadamente a sua escassez e reduzido número de amostras que podem ser avaliadas diariamente.